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- LIV. 1929. P. 366, line 2 from the bottom, for "1133-1141" read "181-189."  
 P. 367, line 6 from the bottom, for "73-81" read "324-333."  
 P. 368, line 7 from the bottom, for "1121-1132" read "169-180."  
 P. 370, line 13, for "975-994" read "23-42."
- Vol. LV. 1930. P. iii. of Index. The last item in the list of errata (P. 007) refers to Vol. LIV, 1929, and not to Vol. LV., 1930.  
 P. 349. In the abstract of the paper by Wagenaar, for "cystine" read "cytisine."  
 P. 371, line 6 from the bottom, for "iron content" read "phosphorus content."  
 P. 747, line 18 from the bottom, for "Bromphenol" read "Bromthymol."
- LVI. 1931. P. 21, line 7, for "1925" read "1926."  
 P. 134, line 15, for "22" read "20."  
 P. 250, line 21, for "2 per cent." read "10 per cent."  
 P. 276, line 34, for "27.30" read "27.83."  
 P. 296, line 15, for "0.2 mgrm." read "0.2 grm."  
 P. 303. The legends beneath the lower block should be reversed: "C" represents silver salicylate; "D" silver benzoate.  
 P. 334, line 4, for "ClO<sub>3</sub>" read "KClO<sub>3</sub>."  
 P. 334, line 5 from the bottom, for "determined from the data . . ." read "determined. From the data. . . ."  
 P. 397, line 4 of the Note. The reference to Lerrigo should read "1930, 433" and not "1931, 433."  
 P. 421, line 19, for "Lamy" read "Lémy."  
 P. 508, line 4, for "L. Marks" read "S. Marks."  
 P. 632, line 15 from the bottom, for "that more than traces of tannin are present" read "that not more than traces of tannin are present."

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# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, December 3rd, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—Kenneth Bullock, M.Sc., Ph.D., and Frederick Cecil Hymas, B.Sc. A.I.C.

Certificates were read for the second time in favour of:—Arthur Nicholls Ainsworth, B.Sc., Bertram Arthur Gough, William Henry Gough, M.Sc., A.I.C., and William Henry Shilling, B.Sc., A.I.C.

The following were elected Members of the Society:—Leonard Balmforth, B.Sc., F.I.C., Reginald Joseph Cole, B.Sc., Violet Dorothy Dudman, B.Sc., A.I.C., Frank George Edmed, O.B.E., B.Sc., A.R.C.Sc., F.I.C., Roy Gardner, D.Sc., F.I.C., William Victor Griffiths, B.Sc., A.I.C., Daoud Younis Haddad, B.Ph., Percy George Terry Hand, F.I.C., Magnus Herd, B.Sc., A.R.T.C., F.I.C., Gilbert Underwood Houghton, B.Sc., A.I.C., Archibald Robert Jamieson, B.Sc., F.I.C., William Jefferys Lesley, M.Sc., Ph.D., A.I.C., Allison Reginald Murray MacLean, B.A., M.Sc., Ph.D., Frederick Henry Newington, F.I.C., Colin Paterson, B.Sc., A.I.C.

The following papers were read and discussed:—"A Storage and Delivery Apparatus for Antimony Chloride and other Corrosive Reagents," by G. Middleton, B.Sc., A.I.C.; "Tests for Impurities in Ether," Parts II and III, by G. Middleton, B.Sc., A.I.C., and F. C. Hymas, B.Sc., A.I.C.; "The Determination of Small Quantities of Calcium in Magnesium Salts," by Norman Evers, B.Sc., F.I.C.; and "A New Method for the Detection of Nitro-Group in Organic Compounds," by P. K. Bose, D.Sc.

### NORTH OF ENGLAND SECTION.

A MEETING of the Section was held on December 6th, the members, by kind invitation, being the guests of the Co-operative Wholesale Society.

In the morning the party, twenty-six in number, met in Manchester, and was conveyed to the C.W.S. works at Middleton Junction, where the processes of

manufacture of malt vinegar, jam, etc., were inspected. The party returned to Manchester and was entertained to lunch.

In the afternoon a meeting was held at which the following papers were read and discussed:—"The Reichert-Polenske-Kirschner Values of Rancid Butters and Margarines," by G. D. Elsdon, B.Sc., F.I.C., R. J. Taylor and P. Smith; "The Detection of Benzoic Acid in Food Stuffs," by A. N. Leather, B.Sc., F.I.C.

Prof. W. H. Roberts opened a discussion on the standards and definitions for jam recently agreed upon by the Food Manufacturers Federation and the Society of Public Analysts. An adjournment for tea was followed by a thorough discussion in which most of the members participated.

## Death.

WITH great regret we record the death, on December 25th, of Dr. Henry Leffmann, who was a Vice-President of the Society in 1901-2.

## The Determination of the Milk Proteins.

By GEORGE M. MOIR, M.Sc., Ph.D., A.I.C.,

*Pedler Research Scholar of the Institute of Chemistry, 1928-1930.*

*(Read at the Meeting, November 5, 1930.)*

### I. THE CHEMISTRY OF THE SEPARATION OF CASEIN.

#### INTRODUCTION.

A CERTAIN amount of confusion has arisen because of the fact that the substances described in continental and American literatures as "casein" and "paracasein" have sometimes been referred to in English literature as "caseinogen" and "casein," respectively. The first term in each case signifies the base-free protein as normally obtained from milk by acidifying, while the second is applied to the modified base-free protein obtained by the action of rennin on milk. The former terminology, which is adopted in this paper, has received much wider recognition, and it would be a great advantage if the term "caseinogen" were dropped.

Casein exists in milk not as a solution but as an ultra-microscopic dispersion of particles which consist of a compound of the protein with calcium, and this compound is often loosely referred to as "casein." It is highly probable that the colloidal calcium phosphate of milk is very closely associated, if not actually combined, with the dispersed protein. This colloidal material undoubtedly plays a

large part<sup>1</sup> in stabilising the fat emulsion of milk in a serum which contains two other proteins, albumin and globulin, which are considered to be actually in solution. It is very likely that a small amount of these two proteins is adsorbed upon the fat globules, thereby assisting the casein to stabilise the emulsion. The possibility, slight though it is, that the soluble proteins may be adsorbed upon the caseinate micelles, must not be overlooked. Owing to the intimate association of the fat and the casein it is impossible to separate the latter from the milk without removing the former at the same time. Consequently, any other protein matter associated with the fat may also be thrown down and remain with the precipitate unless it can be removed.

Some of the earlier methods used for estimating casein in milk (described by Richmond<sup>2</sup>) have long since been abandoned. However, I have used the principle of weighing the fat together with the casein (after drying) as the basis of a method which may be useful for estimating the value of milk for cheesemaking. Some of the other rapid processes for determining the casein after precipitation, such as Van Slyke and Bosworth's titration method,<sup>3</sup> Hart's centrifugal method,<sup>4</sup> Robertson's refractometric method,<sup>5</sup> and the iron alum titration method of Arny and Pratt<sup>6</sup> are, no doubt, capable of giving more or less satisfactory results if properly carried out, but they cannot be expected to yield results as accurate as those based on the nitrogen content of the precipitate. The same can be said of Harris's<sup>7</sup> potentiometric titration method for the total protein in milk.

When salts are used to precipitate the casein, not merely do they lack any solvent effect on the other proteins, but they usually have a tendency to render the latter more insoluble, with the result that unduly high values are obtained. That this is the case with potash alum, which has most frequently been used for casein determination, is clear from the extensive series of figures recently published by Danish investigators.<sup>8</sup> Howe<sup>9</sup> has also shown that saturated sodium chloride precipitates part of the globulin, while it has long been known that magnesium sulphate precipitates the casein and globulin together.

**THE USE OF AN ACID PRECIPITANT.**—The choice of a satisfactory and accurate method for separating the casein must therefore fall on the use of an acid reagent which will remove as much as possible of the material combined or associated with the protein as it exists in milk. Acetic acid has been much used for this purpose in the past, but Van Slyke and Winter<sup>10</sup> found that some latitude was permissible in the amount of acid that could be used with the highest quantitative results; also, that the higher the casein content of the milk (and therefore the greater the buffer value), the greater the amount of acid necessary.

The successful use of acetic acid depends on the fact that casein is a protein which is insoluble at its isoelectric point, which can be roughly attained by mixing definite quantities of milk and acid. Usually slight excess of acid is used to ensure complete precipitation and ease of filtration, but this involves the risk of redissolving some of the casein. This risk is evident from my analytical results, some of which will be given later, and also from the observations of others.<sup>11</sup>

The idea of precipitating the casein from milk as close as possible to its isoelectric point was first put forward by Waterman<sup>12,13</sup>, but his method has proved unsuitable. With this objective it is necessary in the first place to have the  $pH$  of the point as definitely fixed as possible. Investigations have been made by Michaelis and his collaborators, who, as a result of their cataphoresis experiments, first stated the point as  $pH$  4.74.<sup>14</sup> More careful experiments<sup>15</sup> subsequently gave a value of  $pH$  4.6 ( $H^+ = 2.4 - 2.5 \times 10^{-5}$ ). In a later paper<sup>16</sup> Michaelis showed by a series of nephelometric precipitation experiments that the point of optimum flocculation of casein was capable of being very considerably altered by the presence of different salts in the solution from which it was precipitated. In the presence of sodium acetate alone it lay between  $pH$  4.4 and 4.7 (in these particular experiments his technique was developed with differences of 0.3  $pH$ ). In view of the possibility of such variations it is not surprising that Csonka, Murphy and Jones,<sup>17</sup> by solubility determinations in a series of buffer solutions, found the isoelectric point at  $pH$  4.85. A further factor that is likely to cause variation in such determinations is that in the preparation of the "pure" casein employed for the experiments, changes may have been induced in the original material, especially if it has been subjected to a  $pH$  even faintly alkaline.<sup>18,19</sup> Recently Lebermann<sup>20</sup> treated skim milk with acetate mixtures of varied composition, and concluded (from what appear to be rather inadequate experiments) that the optimum precipitation of the casein occurred at  $pH$  4.89.

Waterman<sup>13</sup> pointed out that the amount of acetic acid (1.5 ml. of 10 per cent.) usually used for 10 ml. of milk precipitated the casein at about  $pH$  4.2, which is considerably below its isoelectric point. He recommended treating the milk with a definite amount of an acetate mixture ( $pH$  4.61), so that the casein was precipitated at  $pH$  4.7-4.8. To avoid the necessity of washing the precipitate, and also the prolonged Kjeldahl digestion due to the presence of the filter paper, he determined the nitrogen content of an aliquot portion of the filtrate. The casein nitrogen was then obtained by difference from the total nitrogen content of the milk found by a separate determination. This procedure neglected the volume of the fat, casein and other constituents of the milk, so that low results were to be expected—an expectation which I confirmed by comparison with direct determinations upon the washed casein precipitate. As a matter of fact, the Kjeldahl digestion is prolonged not so much by the filter paper as by the fat which is included in the precipitate.

The buffer mixture proposed by Waterman is not sufficiently acid and the casein is precipitated at too high a  $pH$ , with the result that it does not flocculate properly, but yields a cloudy filtrate. This is another reason for the low values which have been found by me and by others<sup>21</sup> when using his method.

**EXPERIMENTS WITH BUFFER MIXTURES.**—With a view to finding a more suitable buffer mixture, a large number of experiments were carried out, but these can only be briefly mentioned. Owing to the very great natural variations which different samples of milk present to the analyst, and especially because of

the possible complication of souring (partial or complete), which may occur before analysis, it seemed desirable to investigate a number of different milks to see if it would really be satisfactory to propose the use of any particular buffer mixture for all cases. Trials were made of several mixtures containing acetic acid and sodium acetate in different proportions, and the final  $pH$  of the diluted milk mixture (as determined by means of the quinhydrone electrode) was always found to lie within narrow limits. In Table I appear the results obtained with one such

TABLE I.

Milk No.	Titratable acidity per cent. lactic acid. Per Cent.	$pH$ of milk.	$pH$ of filtrate.
1.*	0.153	6.73	4.64
Same allowed to sour	0.43	5.41	4.53
2.	0.165	6.72	4.67
3.	0.165	6.70	4.59
4.*	0.185	6.63	4.68
5.*	0.12	6.89	4.64
6.*	0.153	6.72	4.74
7.	0.153	6.77	4.69
8.*	0.125	7.02	4.74
9.	0.165	6.66	4.73
Same allowed to sour	0.19	6.63	4.67
" " " "	0.475	5.45	4.61
10.*	0.13	7.02	4.70
11.*	0.18	6.72	4.70
12.*	0.128	6.86	4.71
13.*	0.173	6.69	4.64

buffer which was made by mixing 90 ml. of  $N/1$  acetic acid with 35 ml. of  $N/1$  caustic soda, so that the acid and sodium acetate concentrations were respectively 0.44  $N$  and 0.28  $N$ . The  $pH$  of the mixture was 4.42, and 5 ml. (containing 132 mgrms. of free acid) were added to 10 ml. of milk, diluted first to about 50 ml. The majority of the milk samples used (those marked \*) were from individual cows, as these usually provide a greater range of variation than bulk samples.

These results, together with others similarly obtained, showed that a buffer mixture of the type described could be used upon milk of widely varying composition in order to precipitate the casein within a comparatively narrow range of  $pH$  (0.15, excluding soured samples).

COMPARATIVE CASEIN DETERMINATIONS.—When comparative analyses were carried out by combining before adding to the diluted milk the same amount of acetic acid, with gradually increasing amounts of sodium acetate, greater amounts of casein were precipitated as the  $pH$  decreased (Table II). At the same time the opalescence of the filtrate decreased as increasing amounts of nitrogen were precipitated, but the filtrates were never quite clear like those obtained when

the acetic acid alone was used; yet the results from the acid alone were always appreciably low in this and other experiments.

TABLE II.

No.	Amounts used of		pH of filtrate.	Casein N	Average.
	10 Per cent. acetic acid.	0.28 N sodium acetate.		per 100 ml. milk.	
	Ml.	Ml.		Grm.	Grm.
A.i	1.5	—	4.24	0.3836	—
ii	"			0.3804	0.3820
B.i	"	1.5	4.41	0.3985	
ii	"			0.3999	0.3992
C.i	"	3	4.53	0.3915	
ii	"			0.3993	0.3954
D.i	"	5	4.66	0.3910	
ii	"			0.3890	0.3900
E.i	"	8	4.80	0.3875	
ii	"			0.3883	0.3879
F.i	"	11	4.91	0.3835	
ii	"			0.3834	0.3835

A considerable improvement was obtained when, instead of adding the acid and acetate combined, the acid was first added and then the acetate. After being allowed to stand for an hour to attain equilibrium, the filtrates obtained were usually quite clear over a considerable range of pH, provided the acidified mixture had not been too vigorously stirred. Tables III-V contain typical results, which warrant the conclusion that a maximum amount of casein is usually precipitated by acetic acid and sodium acetate at about pH 4.5-4.7—a range which agrees with the isoelectric point of this protein, as determined by Michaelis.

TABLE III.

No.	Amounts used of		pH of filtrate.	Casein N	Average.
	10 Per cent. acetic acid.	0.28 N sodium acetate.		per 100 ml. milk.	
	Ml.	Ml.		Grm.	Grm.
A.i	1.5	—	4.27	0.4476	
ii	"			0.4468	0.4472
B.i	"	1.5	4.43	0.4355	
ii	"			0.4440	0.4398
C.i	"	3	4.55	0.4495	
ii	"			0.4510	0.4503
D.i	"	5	4.68	0.4498	
ii	"			0.4522	0.4510
E.i	"	8	4.80	0.4470	
ii	"			0.4492	0.4481
F.i	"	11	4.89	0.4475	
ii	"			0.4480	0.4478
G.i	"	15	5.00	0.4468	
ii	"			0.4461	0.4465

## MOIR: THE DETERMINATION OF THE MILK PROTEINS

TABLE IV.

No.	Amounts used of		pH of filtrate.	Casein N per 100 ml. milk. Grm.	Average. Grm.
	10 Per cent. acetic acid. Ml.	0.28 N sodium acetate. Ml.			
A.i	1.5	—	4.22	0.3857	
ii				0.3865	0.3861
B.i	„	1.5	4.37	0.3889	
ii				0.3868	0.3879
C.i	„	3	4.51	0.3923	
ii				0.3920	0.3922
D.i	„	5	4.62	0.3886	
ii				0.3892	0.3889
E.i	„	8	4.75	0.3876	
ii				0.3904	0.3890
F.i	„	11	4.86	0.3876	
ii				0.3887	0.3882
G.i	„	15	4.98	0.3872	
ii				0.3881	0.3877

TABLE V.

No.	Amounts used of		pH of filtrate.	Casein N per 100 ml. milk. Grm.	Average. Grm.
	10 Per cent. acetic acid. Ml.	0.28 N sodium acetate. Ml.			
A.i	2.5	—	3.97	0.3718	
ii				0.3687	0.3703
B.i	2.0	—	4.07	0.3844	
ii				0.3816	0.3830
C.i	2.0	—	4.07	0.3750	
ii				0.3795	0.3723
D.i	1.5	—	4.22	0.3966	
ii				0.3960	0.3963
E.i	0.5	—	<u>4.91</u>	0.4007	
ii				0.4028	0.4018
F.i	1.5	1.5	4.37	0.4028	
ii				0.4034	0.4031
G.i	„	3.0	4.51	0.4019	
ii				0.4032	0.4026
H.i	„	5.0	4.63	0.4049	
ii				0.4043	0.4046
K.i	„	8.0	4.77	0.4047	
ii				0.4035	0.4041
L.i	„	11.0	4.87	0.4023	
ii				0.3994	0.4011
M.i	„	16.0	5.03	0.3962	
ii				0.3922	0.3941
N.i	„	22.0	5.14	0.3616	
ii				0.3588	0.3602

In the experiment recorded in Table V, the filtrates of A, B, C, M, and N, were all quite opalescent, so that filtration and washing of these precipitates was very slow, lasting for more than thirty hours. The difference between B and C was due



to the use of a simply folded filter for C instead of the usual fluted folding, with the result that filtration took so long that before washing was complete the precipitate began to pass through the filter. It is noteworthy that E precipitated at  $pH$  4.91 with a small quantity of acetic acid, gave practically the same result as L at 4.87, obtained in a different manner. The agreement of the duplicates (i and ii) was more satisfactory in most of the cases where clear filtrates were obtained.

In these, as in other experiments, only very slightly lower amounts of casein were precipitated at  $pH$  values on either side of the maximum point. The decrease became more definite, however, on reaching the  $pH$  obtained by the use of the usual quantity of acetic acid, which was found, as a rule, to give results 1–2 per cent. low. The slightness of the increase in solubility of casein on either side of its point of maximum flocculation has already been demonstrated by Sørensen and Sladek,<sup>22</sup> who published curves showing the effect of the salt content of casein solutions upon the point of minimum solubility of this protein. It is possible that the varying salt content of milk is the reason for slight variations in the  $pH$  at which the maximum amount of casein can be precipitated.

On the basis of the foregoing experiments (together with others which have yielded similar results) a modification of the A.O.A.C. "official" method (in which only 1.5 ml. of 10 per cent. acetic acid per 10 ml. of milk was used) seems justified. This consists in adding 4.5 ml. of 0.25 *N* sodium acetate (3.4 grms. crystals per 100 ml.) after the acid has been added, in the manner to be described in detail later. The use of these quantities of acetic acid and sodium acetate provides a buffer mixture of the same type as that which gave the results contained in Table I, but slightly modified so as to precipitate the casein from most samples of milk at a  $pH$  as close as possible to 4.6.

The foregoing procedure has been evolved for normal cows' milk. Owing to the different buffering powers of the milk of other animals, slight modifications may be required in the amount of sodium acetate needed to attain a  $pH$  of 4.6. For any particular type of milk the best procedure will be to carry out an experiment similar to that recorded in Table III, but without at first completing the nitrogen determinations. Having thus found approximately the right amount of sodium acetate to bring the  $pH$  to 4.6, the filtrate from a number of different samples of milk treated with the same quantity, should be examined to see if this  $pH$  is regularly attainable. Finally, complete experiments like that set out in Table III should be carried out, extending from  $pH$  4.2 to 5.0, to make sure that the maximum amount of casein is precipitated at  $pH$  4.6.

**SUMMARY.**—1. The chemistry of casein and the state in which it exists in milk have been briefly discussed in order to show the desirability of using an acid reagent for the purpose of its analytical separation.

2. Experiments have been carried out which show that by mixing definite quantities of milk and a suitable acetic acid and sodium acetate buffer solution a  $pH$  close to the isoelectric point of casein can be attained with milk of widely varying composition. Even when the milk has soured somewhat, the effect of this on the final  $pH$  of the mixture is slight.

3. Experiments have shown that in order to precipitate a maximum of casein from milk it is desirable to add the buffer solution in two parts, acid first and then sodium acetate. When this is done maximum casein values for milk are obtained between pH 4.5-4.7.

This paper represents part of the work carried out during my tenure of the Pedler Research Scholarship of the Institute of Chemistry, the assistance of which and the interest of the members of the Pedler Fund Committee is gratefully acknowledged. I should also like to add my appreciation of the facilities made available to me at the National Institute for Research in Dairying, and especially of the valuable advice of Capt. J. Golding, Head of the Chemical Department.

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(Part II. *The Identity of the Casein Precipitate will be published in the February issue.*)

## The Determination of Unsaponified Oil in Soap or Fatty Acids.

By E. LESTER SMITH, M.Sc., A.I.C.

(Read at the Meeting, October 1, 1930.)

DURING the manufacture of a concentrate of fat-soluble vitamins by extraction of the unsaponifiable fraction from fish liver oils, it became necessary to have available a rapid method of detecting, and, if possible, of approximately determining, unsaponified oil in soap. Any oil which escapes saponification appears in the final extract and renders it unfit for use, besides causing difficulties in manufacture; re-saponification is troublesome, and may cause destruction of vitamin A. As

little as 0.1 per cent. of unsaponified oil in the soap corresponds to about 10 per cent. of oil in the unsaponifiable fraction of cod-liver oil, which is enough to be objectionable. The test should therefore be a sensitive one. Four methods have been so far available:—

- (1) The apparent amount of unsaponifiable matter will be affected by any unsaponified oil in the fatty acids. This can be quantitatively determined, given a careful quantitative extraction and a knowledge of the unsaponifiable content of the oil. If an accurate method is employed, 0.05 per cent. of unsaponified oil is detectable (*cf.* ANALYST, 1928, 53, 632).
- (2) A test, known as the "Sodium Ethylate Test" has been devised in this laboratory and used as a routine test for the presence of saponifiable oil in concentrates of unsaponifiable matter. It involves a rough extraction of at least 50 grms. of the soap with ether, and the removal of the bulk of the sterols present by filtering an ice-cold alcoholic solution of the unsaponified matter. The sterol-free unsaponifiable matter, after removal of solvent, is tested as follows:—The material (0.2 ml.) is mixed with 0.2 ml. of 2*N* sodium ethylate in a small test-tube, and warmed in a water-bath at 70° C. for two minutes. The tube is then removed and cooled under the tap for a further two minutes, the appearance of the contents being noted. Any oil present is saponified, the soap causing a thickening of the mixture. The following grades are recognised:—(a) No thickening; (b) slight thickening on cooling (produced by 5 per cent. of oil); (c) setting to spongy mass (produced by 7.5 per cent. of oil); (d) setting solid in water bath (produced by 10 per cent. of oil). This test is sensitive to about 0.05 per cent. of oil in the fatty acid, but is tedious to carry out, except when examining final concentrates.
- (3) The acid value of the fatty acids is reduced below the normal value by the presence of neutral oil. The course of a saponification may conveniently be followed by determinations of the acid value of the mixture of oil and fatty acids liberated on acidifying a portion of the saponification mixture. It is scarcely sensitive enough, however, to distinguish between 99.5 per cent. and 100 per cent. saponification, *i.e.*, to detect less than 0.5 per cent. of oil in the fatty acid.
- (4) Lewkowitsch ("The Examination of Oils, Fats and Waxes" (1921), Vol. I, p. 112), describes the following "emulsion test":—Three c.c. of the fatty acid are mixed with 15 c.c. of 95 per cent. alcohol and 15 c.c. of concentrated aqueous ammonia; turbidity indicates unsaponified oil. Rutzler (*Oil and Fat Ind.*, 1929, 6, [No. 9], 23; also, *Soap*, 1930, 4, [No. 5], 31), has recently re-investigated this test and proposes a modification which renders it more sensitive. He found, when using the proportions Lewkowitsch suggests, that coconut oil fatty acids of low unsaponifiable content gave no cloudiness when they contained less than 1 per cent. of oil. He.

therefore, proposes to add 1.2 per cent. of mineral oil to the fatty acid; this produces a turbidity perceptibly increased by 0.076 per cent. of coconut oil. The turbidity is measured in an "emulsometer," the depth of liquid in a Nessler glass being varied until the light of a lamp, shining through a pinhole at the end of a long iron tube beneath the Nessler glass, is just extinguished. It is necessary to standardise the time for which the mixture is allowed to stand, since turbidity varies with age.

Lewkowitsch's test is very insensitive, and Rutzler's modification is still not accurate enough. An examination of the principles on which the test is based, however, has suggested modifications which increase the sensitiveness of the test at least tenfold, without the use of mineral oil. Cod-liver oil fatty acids have been used as the test material, and every possible factor varied independently over wide limits to discover the conditions of maximum sensitivity. As a basis 1 gm. of fatty acid, containing 0.1 per cent. or less of cod-liver oil was used for the test, 1 ml. of aqueous ammonia of sp. gr. 0.880 diluted with an equal volume of water, instead of the enormous excess used by Lewkowitsch and Rutzler, and various volumes of mixtures of alcohol and water of different compositions. It was soon found that no turbidity was produced in very strong alcohol, as might be expected, owing to the distinct solubility of oil in this medium, or in water or weak alcohol; maximum turbidity was produced with aqueous alcohol containing about 65 per cent. of alcohol by volume. Moreover, strong solutions of the ammonium soap, of the order of 10 per cent., as used by Lewkowitsch and Rutzler, gave no turbidity; this appeared, however, on dilution with spirit of the same strength; the maximum appeared at about 1.7 per cent., *i.e.*, 1 gm. of fatty acid in 60 ml. of solution. Equivalent amounts of sodium hydroxide or potassium hydroxide in place of ammonia produced no turbidity with small percentages of oil, and slowly destroyed the turbidity present if added to the ammoniacal solutions. Triethanolamine gave a turbidity of about the same intensity as ammonia. Varying the ammonia between 0.5 ml. and 2.0 ml. per gm. of acid made no noticeable difference. Under no conditions was the slightest haze observed with fatty acid free from oil, except after standing overnight, despite the presence of over 1 per cent. of unsaponifiable matter therein. Alterations in the order of mixing the reagents had no appreciable effect. With very small percentages of oil, the haze did not appear until after standing for some minutes, and gradually increased, even after some hours. Solutions could usefully be compared after fifteen minutes.

These observations may best be explained as follows:—The oil is always thrown out in the form of a finely dispersed emulsion when it is insoluble in the test solution; when no haze appears, the oil is either in molecular or colloidal solution; the former is the case in strongly alcoholic solutions; in solutions in water or dilute alcohol, and when the soap concentration is high, the oil is "dissolved by the soap," or adsorbed by the colloidal soap micellae. The ideal conditions, therefore, are those in which the soap is mostly in crystalloid form, *i.e.*, in dilute solution in strong alcohol, except that the alcohol must not be so strong as to dissolve the oil

appreciably, nor the dilution so great that the haze is invisible. Soda and potash give no turbidity, because the excess alkali present saponifies the trace of oil. If the exact equivalent of these alkalis is employed, turbidity occurs, as with ammonia, which is not a sufficiently powerful alkali to saponify the oil. The unsaponifiable matter present gives no turbidity, owing to its solubility in alcohol of the strength employed. In order to show that unsaponifiable matter has no great effect on the modified test, mixtures of synthetic glyceryl oleate with pure oleic acid, free from unsaponifiable matter, were tested; 0.05 per cent. of olein in the mixture gave a just perceptible haze, and 0.1 per cent. a marked cloudiness. Similarly, 0.05 per cent. of olive oil in pure palmitic acid, could be detected. At the other extreme the fatty acids from a "technical" fish-liver oil containing 6.7 per cent. of unsaponifiable matter gave only a slight haze, which was markedly increased by 0.1 per cent. of oil added to the fatty acid. Slight turbidity is best detected by looking through a column of the liquid in a Nessler cylinder held above a dark surface, in a good light. If the solution is illuminated with a beam of light in a dark room, a Tyndall cone effect is observed when oil is present, but this method of examination does not appear any more sensitive than the above; it is very easy to determine by inspection whether a liquid is quite bright or not.

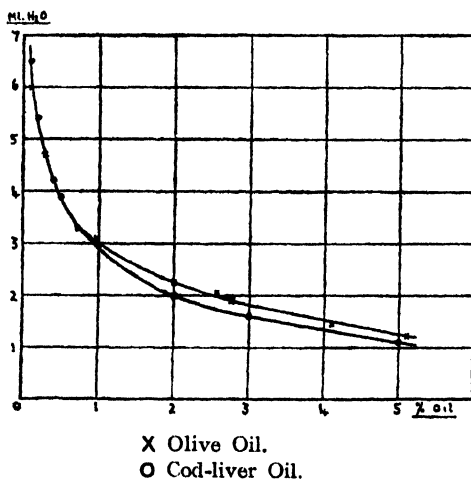
**METHOD OF USING THE TEST.**—The procedure finally adopted for the test is as follows:—The soap is decomposed by boiling with dilute hydrochloric acid, and the fatty acid skimmed off without any attempt to dry or otherwise to purify it. One grm. is weighed into a flask, melted if necessary by gentle warming, and 60 ml. of 65 per cent. alcohol (by volume) and 1 ml. of ammonia (0.880 sp. gr., diluted with water, 1:1) added. At the same time a blank is prepared with pure fatty acid. The comparison is made as described above, after allowing the flask to stand for at least 10 minutes.

It was found in the case of cod-liver oil that 0.025 per cent. of oil in the fatty acid could just be detected. In the original Lewkowitsch test 0.5 per cent. of oil gives the faintest perceptible haze. Quantities of oil of 0.1 per cent. or more give with the modified test a cloudiness so obvious that no blank is necessary. This test is, therefore, at least as sensitive as any available, besides being simpler and quicker. We have found the sensitiveness to be of the same order for all natural oils examined, and practically independent of the amount of unsaponifiable matter, unless this is excessive, contrary to Rutzler's suggestion (*loc. cit.*). Mineral oil, however, gives a turbidity in the same way as vegetable oil.

Attempts to devise a quantitative test, which shall be independent of the nature of the oil, have not proved so successful. Nephelometric estimation of the turbidity is so complicated by the colour of the solution, by the dependence of degree of turbidity on time of standing, and by the considerable range of oil concentration over which the test can be used, that this method was abandoned. The emulsometer described by Rutzler gives surprisingly concordant results, considering its simplicity, and can be recommended for quantities of oil above 1 per cent. Below this, the column of liquid necessary to extinguish the light becomes impossibly long. Smaller quantities can, of course, be estimated by adding an extra

0.5 per cent. or 1 per cent. of oil to the fatty acid to be tested, but the accuracy is not greater than about  $\pm 0.1$  per cent. Results are not independent of the nature of the oil, so that a separate calibration curve must be constructed for each oil. Very small amounts of oil are best determined by comparison with standards made from pure fatty acids to which known percentages of the oil have been added; the solutions must be freshly prepared at the same time as the test solution.

**QUANTITATIVE METHOD.**—The test may be rendered quantitative by another modification. If 0.5 grm. of the fatty acid is dissolved in 20 ml. of 99 per cent. alcohol, and 0.5 ml. of ammonia added, no turbidity appears. If water is now added from a burette, the volume required to produce incipient turbidity is some indication of the amount of oil present. Unfortunately, the volume required is not independent of the speed at which it is added, which must be standardised in some way. The method finally evolved is as follows:—Two-and-a-half grms. of the fatty acid are weighed into a 100 ml. graduated flask and dissolved in alcohol, 2.5 ml.



of ammonia are added (0.880 sp. gr., mixed with an equal volume of water), and the solution is made up to the mark with alcohol. It is important to standardise the strength of alcohol used. Four 20 ml. samples are pipetted into test-tubes of suitable size; the 20 ml. (approx.) remaining are titrated in the flask with water to incipient turbidity, to give an indication of the amount required; to the four tubes are added volumes of water slightly less than the volume required to give immediate turbidity, and varying by 0.1 or 0.2 ml. After having stood for 5 minutes the tubes are compared, and the volume of water which has just produced opalescence under these conditions is noted. It is necessary, of course, to prepare a calibration curve, using standard mixtures of oil and fatty acid, and the amount of oil present can then be read from the curve..

It was hoped that the curve would be independent of the nature of the oil and fatty acid; olive oil and cod-liver oil, mixed with their corresponding fatty acids, were compared, and gave nearly identical calibration curves, as shown in the Figure.

For most practical purposes, *e.g.*, in the soap industry, it would be sufficient to prepare such a curve for a typical soap stock, and to assume its validity for other oils. The method is simple and fairly rapid, and has the further advantage that its sensitiveness increases as the proportion of oil decreases. It is suitable for amounts of oil between 0.1 per cent. and 10 per cent.

SUMMARY.—Modifications of the "emulsion test," in which the presence of oil in fatty acid is indicated by the turbidity of a solution of the ammonium soap in dilute alcohol, are described.

As little as 0.05 per cent. of oil can be detected by the modified test, which is rapid and as sensitive as any other available.

The test may be rendered quantitative by a determination of the volume of water required to produce incipient turbidity in an alcoholic solution of the ammonium soap under standard conditions.

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## The Determination of Phosphorus in Steel, Alloy Steels and Cast Iron.\*

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PHOSPHORUS is oxidised to orthophosphoric acid in nitric acid solution and precipitated as ammonium phospho-molybdate, which was at one time weighed, but is now usually determined titrimetrically. The process is a very old one, the condition necessary for the formation of the precipitate having been investigated by Hundeshagen (*Z. anal. Chem.*, 1889, **28**, 141). Technical books on steel analysis show wide variations in describing the method as regards concentration of reagents, etc., as pointed out by Ridsdale (*Proc. Cleveland Inst. Engineers*, 1919, p. 155), most of which give fairly good results for medium or high phosphorus, but not all give correct figures for low phosphorus, or when arsenic is present. (High phosphorus = 0.1 per cent. or more; medium phosphorus = 0.05 per cent.; low phosphorus = 0.02 per cent. or less.)

The following is the method which I have used for some years and have found to be accurate even down to 0.002 per cent. of phosphorus in electrolytic iron. It is applicable to plain carbon steels, wrought iron and pure iron. Two grms. of steel are dissolved in a 300 c.c. Erlenmeyer flask in 45 c.c. of nitric acid (sp. gr. 1.2), and gently boiled for 5 minutes to oxidise carbides. Several drops of saturated

\* Communication from the Research Department, Woolwich.

potassium permanganate solution are added to the boiling solution until a permanent precipitate of manganese dioxide is formed. After boiling gently for 5 minutes this precipitate is dissipated by careful addition of sodium nitrite crystals and freed from oxides of nitrogen by boiling for a short time. After cooling, 10 c.c. of dilute ammonia (1:1) are added, and the liquid shaken well to re-dissolve any iron hydroxide precipitated on the sides of the flask. The liquid is now heated to 80° C. (registered by a thermometer in the flask), the flask taken from the source of heat, 30 c.c. of molybdate reagent added, and the liquid well shaken to assist in the formation of the precipitate.†

After standing 10 minutes the precipitate is filtered on a pulp filter, washed twice with 5 per cent. nitric acid and six times with 5 per cent. potassium nitrate solution to wash all acid away (the washings are tested with litmus paper). The pulp and precipitate are placed in a beaker, 50 c.c. water added, and the mixture well shaken to disperse the precipitate through the liquid. Twenty c.c. of sodium hydroxide solution are now added from a pipette, and the flask well shaken.‡ If this does not dissolve the precipitate, as shown by the yellow colour disappearing (high phosphorus) a further 20 c.c. is added. It is very important to shake well to bring every particle of precipitate into contact with the alkali. The excess of alkali is now titrated with standard sulphuric acid, phenolphthalein being used as indicator.

The value of 20 c.c. of sodium hydroxide solution is found in terms of sulphuric acid by a separate titration in the presence of 50 c.c. of water. Thus, the phosphorus is expressed as a number of c.c. of the sulphuric acid, the exact value of which, in terms of phosphorus, must be found once for all. It is convenient to make up a large volume of this acid (about 5 litres) of approximately *N*/10 strength. It is standardised by a series of tests with standard sodium phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ ) solution (1 c.c. = 0.001 gram. phosphorus) made from A.R. clear crystals and measured from a standard burette. In these experiments 2 grms. of electrolytic iron are added, and after the addition of the sodium phosphate solution, water and nitric acid (sp. gr. 1.42) are added to make 45 c.c. of nitric acid (sp. gr. 1.2), as in the method. It is convenient to use 10, 12 and 14 c.c., equivalent to 0.05, 0.06 and 0.07 per cent. of phosphorus, and to make a blank experiment with electrolytic iron alone. After correcting for the phosphorus in the electrolytic iron (usually 0.002 per cent.), the value of the acid, in terms of phosphorus, is calculated.

A few experiments conducted with the sodium phosphate in a similar manner, but in which the phosphorus is determined by the lead molybdate gravimetric process, are usually put through as a check on the sodium phosphate, and have always been found to agree. The sulphuric acid can also be standardised titrimetrically against hydrochloric acid (*N*/10), which has been standardised by the

† The molybdate reagent is made from ammonium molybdate, 160 grms., dissolved in a mixture of 300 c.c. of water and 120 c.c. of ammonia (sp. gr. 0.880), poured slowly, with stirring, into 1500 c.c. of nitric acid (sp. gr. 1.2).

‡ The sodium hydroxide solution is made from the solid sticks purified by alcohol and also contains barium to keep it free from carbonate. A soda-lime tube attached to the stock bottle prevents access of  $\text{CO}_2$ . The solution is approximately *N*/10 strength.



gravimetric silver chloride process. This titrimetric factor is based on the following equation:



This factor has been found to agree with the factor above.

NOTES ON THE PROCESS.—There is some danger of loss of ammonia at the stage where 10 c.c. of ammonia (50 per cent.) are added, if the liquid is not properly cooled, or if the ammonia is added too quickly.

It would be possible to add ammonium nitrate at this stage, but this would require less nitric acid to be used in dissolving the steel, which would be undesirable for rapid solution and oxidation of the carbides formed.

Clear crystals of sodium phosphate are used, not those which show signs of efflorescence, in order to ensure the correct composition, *viz.*  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ .

Oxidation with permanganate is necessary to destroy organic matter formed from carbon in the steel which is not completely decomposed by nitric acid. The omission of permanganate leads to low results, possibly due to the interference of organic substances with the formation of the yellow precipitate. The precipitate should not stand longer than 10 minutes, owing to the possibility of co-precipitation of arsenic (see later). From the equation given it will be observed that  $1\text{P} = 23\text{NaOH}$ , and hence the titration is capable of great accuracy, owing to the small titration factor. In fact, 1 c.c. of *N*/10 sulphuric acid equals 0.000135 gm. of phosphorus, equals 0.00675 per cent. on 2 grms. taken, so that errors in titration are almost negligible.

The agreement shown between the sodium phosphate method and the titrimetric method based on the equation given above proves the accuracy of the alkali-metric titration. This question was investigated by Nyssens (*Bull. Soc. Chim. Belge*, 1925, p. 232). He shows that there is no appreciable error in the use of phenolphthalein as indicator, although the ammonium radicle derived from the precipitate is present, so that it is not necessary to add excess of alkali and boil out the ammonia (as recommended by Richards and Godden for large precipitates, *ANALYST*, 1924, 49, 565). This has been confirmed by Cameron and Dow (*ANALYST*, 1927, 52, 576), who consider that it is also accurate for larger precipitates than obtained in steel analysis. Nyssens (*supra*) also showed that it is satisfactory to use the theoretical titrimetric figure for the standard acid, based on the equation given.

The metallurgical analyst is to-day ever increasingly called upon to determine phosphorus in alloy steels, in which it is not always possible to apply the standard procedure which has been described. In these cases it has been my aim to obtain the same conditions in the solution, prior to precipitation with molybdate, as exist in the standard method. Modifications which require the presence of ammonium chloride or sulphate, are undesirable. Ammonium chloride favours co-precipitation of arsenic, and ammonium sulphate hinders the formation of the yellow precipitate (this is markedly the case with low phosphorus steels)—hence, sodium nitrite is preferred to sulphurous acid in reducing manganese dioxide.

Also, it is by no means certain that the yellow precipitate has exactly the same composition with varying conditions, which is not unlikely in the case of such a complicated molecule.

**MODIFICATIONS WITH PLAIN CARBON STEELS.**—Before considering the alloy steels, etc., there is one modification which may be necessary in certain cases with plain carbon steels. This is due to the presence of arsenic, which is found in practically all steel. Usually arsenic is present only to the extent of about 0.03 per cent., and also if the phosphorus is not greater than 0.05 per cent., there is, in these circumstances, no co-precipitation. I have investigated the matter, and have found that with increasing phosphorus there is a slight but appreciable co-precipitation, even with 0.02 per cent. of arsenic, and the higher the phosphorus the greater is the tendency. With high arsenic content (*e.g.* 0.10 per cent.) and phosphorus below 0.02 per cent., the tendency is very small, but beyond that point results are appreciably too high. The outcome of the work is that it is recommended to use a modified process where the ordinary method has given results greater than 0.05 per cent., unless it is independently found that the arsenic is less than 0.02 per cent., and in any case, if arsenic is greater than 0.10 per cent.

In the modified process the arsenic is removed. The ordinary process is followed as far as the precipitation of phosphomolybdate and washing with nitric acid, and one wash with potassium nitrate. From this point the process is as follows:—The precipitate is dissolved in 20 c.c. of ammonia (1 : 1), and after well washing the pulp with water, hot dilute hydrochloric acid (1 : 1) is run through the pulp, which is again washed with water. An excess of hydrochloric acid is added to the liquid, sufficient to react well with zinc dust, of which about 0.5 gm. is added. This reduces arsenic to the arsenious condition (also eliminates some as arsine). The liquid is now saturated with hydrogen sulphide and the flask closed and allowed to stand over-night. Arsenic is precipitated as  $As_2S_3$ , with some of the molybdenum as  $MoS_3$ . The precipitate is filtered and washed with ammonium chloride solution. The filtrate is boiled to drive out hydrogen sulphide and oxidised with nitric acid (sp. gr. 1.2 per cent.), which contains 0.1 gm. of electrolytic iron, previously dissolved in it.

After cooling a little, the iron is precipitated by ammonia in excess, which carried down all the phosphorus. This precipitate is filtered off, washed well with hot water and dissolved in 45 c.c. of nitric acid (sp. gr. 1.2), and the filter paper well washed. To the liquid is now added 1.9 grms. of electrolytic iron, and when this has dissolved the liquid is boiled down to 45 c.c., during which process practically only water is lost, owing to the fact that the constant-boiling mixture is approximately of 1.2 sp. gr.; in other words, the original condition has been arrived at. The ordinary process is now followed, and it only remains to correct for the phosphorus in the electrolytic iron (usually 0.002 per cent.).

This method has been tested both with sodium phosphate and standard steels, after adding 0.10 per cent. arsenic, and has given accurate results.

**CAST IRON.**—In regard to cast iron, there are two classes to be considered, *viz.* high-phosphorus cast iron (pig iron) and low-phosphorus cast iron (haematite

iron or haematite pig). In the high-phosphorus iron it is sufficient to take 0.25 gm. together with 1.75 grms. of electrolytic iron, and to use the ordinary process, ignoring graphite. The correction for the electrolytic iron is of no account in this case. With low-phosphorus cast iron it is necessary to take 2 grms. and to follow the ordinary process, ignoring graphite.

**TITANIUM.**—In these irons there is one element sometimes present which may cause trouble, *viz.* titanium. This was investigated by Ridsdale (*Proc. Cleveland Inst. Engineers*, 1920, p. 109), who showed that if titanium were present to the extent of 0.1 per cent., it seriously interfered. Titanium prevents or hinders the precipitation of phosphorus (for some obscure reason), as has been known for a long time. In fact, the technical books describe a process for overcoming the difficulty which Ridsdale (*loc. cit.*) calls the "long" process—an allusion to the many operations involved. He has improved on this by the use of cupferron, a full description of which is given (*loc. cit.*).

I have modified the method somewhat, preferring to collect the phosphorus in an iron precipitate, following the lines given in the discussion on arsenic, so as to apply the standard procedure for the final precipitation. The bulk of the iron is removed in the first place by the ether-extraction method rather than by the process given by Ridsdale, after which titanium (and the remainder of the iron) is removed by cupferron. It is fortunate that it is not usual to encounter low phosphorus iron with much more than 0.01 per cent. of titanium, which amount is too small to interfere with the ordinary process. With high-phosphorus irons, titanium is rarely of any consequence, since only 0.25 gm. is used.

**SILICON.**—Another element in low-phosphorus iron which has to be considered is silicon. It is convenient to consider high-silicon steels (2 or 3 per cent. of silicon) at this stage. On dissolving the iron in nitric acid a large amount of the silicon is thrown out of solution. If, however, this is filtered off, it will be found that more silicon is precipitated during subsequent operations, seriously interfering with the filtration and washing of the yellow phospho-molybdate. There is also a possibility of silico-molybdate being co-precipitated, owing to the nitric acid solution being saturated with it. It has been found possible to remove the silica, practically quantitatively, from nitric acid solutions by dissolving as usual, adding about 6 drops of hydrofluoric acid (A.R. 40 per cent.), and boiling. Silicon fluoride is formed and hydrolyses at once; more silicon fluoride is formed and so on, the hydrofluoric acid thus acting catalytically. After filtering, washing and boiling down, no further trouble occurs in the ordinary operations.

If considered desirable, traces of phosphorus can be recovered from the silica by washing it off the paper into a platinum dish and evaporating down with hydrofluoric and nitric acids, driving off hydrofluoric acid by repeated evaporation with nitric acid, and finally taking up with a very small amount of dilute nitric acid and adding to the main filtrate.

**ALLOY STEELS.**—Nickel steels, nickel-molybdenum steels, nickel-chromium steels with low chromium and carbon, and manganese steels, give no trouble, and

the ordinary process can be followed. Chromium steels with low chromium (*i.e.* not greater than 3 per cent.) and medium or high carbon show a black residue (chromium carbide) on dissolving, and it is necessary to digest **without** losing much acid till this residue is dissipated. Otherwise no trouble is experienced and the ordinary process applies. It is a different matter with high chromium steels (15 to 20 per cent. chromium), the now well-known and much-used stainless irons and steels, heat-resisting and incorrodible steels, since these will not dissolve in nitric acid of any strength. Some of them do not dissolve at all quickly in *aqua regia* either; and furthermore, those with anything more than a low carbon content throw out a large quantity of carbide which is very resistant to the acid mixture, even on long digestion.

When silicon is also a constituent (2 or 3 per cent.) the position is worse, since the steel itself is often not completely attacked, owing to the particles being coated with gelatinous silica. Hydrochloric acid alone is the best solvent for these steels. However, this is obviously dangerous to use in this determination, owing to possible loss of hydrogen phosphide, but this is overcome by the use of the special apparatus described by Evans (ANALYST, 1929, 54, 286). Hydrochloric acid is used to dissolve the steel (about 20 c.c. HCl and 20 c.c. of water for 2 grms. of steel), and brominated hydrochloric acid is used in the cylinder instead of nitric acid, in order to absorb any hydrogen phosphide which might be evolved.

As a matter of fact, some experiments which have been made appear to show that no loss of phosphorus occurs in hydrochloric acid alone, but this might not always be the case with different steels. When the steel has dissolved and the contents of the cylinder have been drawn over into the flask, as described by Evans, nitric acid is added to oxidise ferrous iron to ferric, and the liquid digested till the carbide has disappeared. The carbide which is thrown out by hydrochloric acid is much easier to dissipate than that given by *aqua regia*, which is another advantage in the use of hydrochloric acid instead of *aqua regia*. The same method is used for nickel-chrome alloys (*e.g.* nichrome wire), except that concentrated hydrochloric acid is used to dissolve the material, which is attacked too slowly by the 50 per cent. acid.

The contents of the flask are now transferred to a beaker and evaporated to dryness (but not baked), taken up with concentrated nitric acid (sp. gr. 1.42), and again evaporated to dryness. This is done several times to eliminate hydrochloric acid as completely as possible, and the residue is finally taken up with 25 c.c. of dilute nitric acid (sp. gr. 1.2) and 20 c.c. of water, since calculation has shown that this is approximately the equivalent of dissolving 2 grms. of steel in 45 c.c. of dilute nitric acid (sp. gr. 1.2), so that the ordinary process can now be followed.

It is necessary to use a very much larger amount of potassium permanganate to get permanent manganese dioxide, since all chromium must be oxidised to chromate first, and experiments appear to show that chromic acid is not effective alone (presumably in oxidising organic matter still remaining, but this point has not

yet been cleared up). It is convenient to add solid permanganate in small amounts at first, afterwards using the saturated solution as usual. It is necessary to use more sodium nitrite also, in order to reduce the chromate back to the trivalent condition, as chromate interferes with the formation of the yellow precipitate, causing too low results.

**VANADIUM AND TUNGSTEN STEELS.**—Vanadium interferes, partly by preventing the complete precipitation of phosphorus (like titanium), but also it is partly precipitated as a complex of similar constitution (like arsenic), recognised by the red colour imparted to the precipitate. These two effects have a compensating action, so that it is possible to obtain correct results by compensation with alloys of certain composition. Many steels are made with not much more than 0.2 per cent. vanadium, and this is too small to affect the result, so that in these cases the ordinary method can be used.

The colour of the precipitate is a criterion as to whether the ordinary method is applicable or not. As regards modifications of methods, those in which vanadium is reduced to the tetravalent condition and phosphorus precipitated at ordinary temperature have been suggested, but have not proved satisfactory. The method suggested by Johnson (*Analysis of Special Steels*, 1920, p. 41) is satisfactory, in which a large excess of nitric acid is used, and the precipitate allowed to stand for some time. Under these conditions vanadium is not precipitated. This is the only case where it is not possible to apply the standard procedure, since there is no satisfactory method for separating phosphorus and vanadium in the presence of a large amount of iron.

Johnson's method gives satisfactory results, tested with standards with or without vanadium added (several hours are required for precipitation). Tungsten interferes if tungstic oxide is precipitated in the ordinary method. Many steels are made with about 3 per cent. tungsten, which does not precipitate in the ordinary process and no trouble arises. With higher tungsten amounts (e.g. tool steels with 15 per cent.) some, but not all, of the tungsten is precipitated, and this always carries some phosphorus. Hence it is necessary to remove tungsten as the oxide and recover the occluded phosphorus.

A method of doing this was devised by me some years ago. It consists in obtaining tungstic oxide quantitatively from 2 grms. of steel by the ordinary hydrochloric acid method, dissolving the oxide in ammonia, and refiltering. The undissolved part appears to contain ferric phosphate and, in my opinion, all the occluded phosphorus is in this form, since attempts to recover more phosphorus from the clear ammoniacal filtrate by acidifying and addition of ferric nitrate (from electrolytic iron) and making alkaline with ammonia, have not yielded any more phosphorus.

It is, perhaps, safer to add a little ferric nitrate to the slightly acidified tungstate filtrate in the first place (the acid being run through the filter to dissolve the iron ferrous phosphate suspension remaining on it), and to precipitate with ammonia. The precipitates containing the recovered phosphorus are dissolved in

hydrochloric acid and added to the main filtrate. With high-tungsten steel it is difficult to eliminate tungsten, owing to the tendency to formation of ferric tungstate, which is carried down in the iron precipitate. A better plan is to pour the acid solution (if very slightly acid tungsten is not precipitated—nitric acid is better than hydrochloric acid in this respect) slowly into the excess of dilute ammonia. This method is very similar to that given by Rooney and Clark (*J. Iron & Steel Inst.*, 1925, 1, 457). The hydrochloric acid is now replaced by nitric acid in the manner already given for stainless steels, and the ordinary method finally applied, or Johnson's method may be used if vanadium is present. It is necessary to remove the tungsten quantitatively in high-tungsten steels. It is far from being completely precipitated in nitric acid; and if the precipitate formed is filtered off, more tungsten is precipitated in the subsequent operations. Hydrochloric acid is necessary for quantitative precipitation.

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## The Analysis of Ferro-Silicon.

BY G. H. GOODWIN, A.I.C.

THE primary object of this investigation was to examine the sources of error in the determination of silicon in high grade ferro-silicons, particularly those containing about 50 per cent. silicon. It is conceivable, however, that the information contained herein will be of use whenever the accurate estimation of silicon or silica is a *sine qua non*.

The chief sources of error in the analysis of silicon alloys (or silicates) are:

- (1) The solubility of freshly precipitated silicic acid in sodium chloride.
- (2) The solubility of freshly precipitated silicic acid in hydrochloric acid.
- (3) Minor errors which can usually be avoided, such as (a) contamination from beakers, dishes, and reagents used, (b) loss of silica during ignition, (c) the absorption of moisture by the silica during weighing, (d) the effect of dehydration at too high a temperature, (e) incomplete dehydration.

Of these, (1) is probably the most important, as a comparatively large weight of flux is necessary. The effect of sodium chloride on the solubility of silicic acid has been investigated by A. B. Trickett (J. W. Mellor, *Treatise on Quantitative Inorganic Analysis*, 1913, 174), and attention has also been directed to it by others, e.g. V. Lenher and E. Truog (*J. Amer. Chem. Soc.*, 1916, 38, 1050), and F. G. Hawley (*Eng. Min. J.*, 1917, 103, 541).

The second error can be made negligible by using dilute acid and avoiding prolonged digestion in the hot or boiling solution.

After precautionary measures have been taken to eliminate the other sources of error the results tend to be too low.

The standard procedure, after fusion with sodium carbonate, evaporation and dehydration, is to filter off the silica and repeat the evaporation and dehydration of the filtrate, which contains the whole of the sodium chloride originally present in the solution.

It has been found, however, that on the addition of ammonium hydrate to the first filtrate, the remaining silica is completely precipitated, *provided sufficient ferric chloride is present in the solution*. The precipitate is then filtered off, washed and redissolved in hydrochloric acid, so that the subsequent operations are carried out in a solution free from sodium chloride.

The same process has previously been used for iron by A. Stadelé (*Arch. für Eisenhüttenwissen.*, 1929, 425), while a somewhat similar one is that employed in Berzelius' and Rose's method for the estimation of fluorides in which silicic acid is precipitated by an ammoniacal solution of zinc oxide (*v. J. J. Berzelius, Pogg. Ann.*, 1824, 1 169, and H. Rose, *ibid.*, 1850, 79, 115). W. F. Hillebrand and G. E. F. Lundell (*Applied Inorganic Analysis*, 1929, 722) also point out that traces of silica left in solution, after a double evaporation to dryness with an intervening filtration, "can be recovered later if an ammonia precipitation is made and a fair-sized precipitate is obtained," *i.e.* when the material under analysis contains appreciable quantities of (mainly) iron or aluminium. As, however, the process has not apparently been applied to the determination of silicon in ferro-silicons, the conditions necessary and limiting values of it have been determined by making up a number of "synthetic" ferro-silicons from "pure precipitated silica" and "Armco" iron drillings.

**DETERMINATION OF SILICA AND SILICON IN MATERIALS USED.**—The first step was to determine the actual percentage of silica and silicon in the materials used.

**Pure Precipitated Silica.**—The specimen contained about 13 per cent. of moisture, and hence it was ignited before use. One grm. of the ignited material was evaporated to dryness with sulphuric and hydrofluoric acids in a platinum crucible, and the residue ignited. The loss in weight gave 98.74 per cent. of silica. The residue in the crucible was fused with a weighed quantity of sodium carbonate, and the silica recovered amounted to 0.08 per cent. After subtraction of the blank for the weight of sodium carbonate taken, the final figure was 98.79 per cent.

**"Armco" Iron.**—The silicon as determined in platinum vessels was 0.002 per cent., and it was consequently ignored. The required weight of drillings was dissolved in hydrochloric acid, oxidised with nitric acid, taken to dryness, redissolved in hydrochloric acid and the solution used without filtration.

In the first series of experiments, 0.25 grm. quantities of the ignited silica were fused with 3 grms. of sodium carbonate, and the cake, after extraction, was decomposed by aqueous hydrochloric acid; varying weights of iron as chloride were

then added. The solution was made alkaline with ammonia, boiled and filtered, and the amount of silica passing into the filtrate determined as follows:

The solution was evaporated to dryness, and the residue baked for two hours at  $110^{\circ}\text{C}$ . The residue was taken up with 10 c.c. of hydrochloric acid (sp. gr. 1.1), warmed for a few moments on a water bath, diluted with cold water and filtered immediately, the precipitate being washed with cold 1 per cent. hydrochloric acid and treated as usual with sulphuric and hydrofluoric acids.

Experiments were then made to determine the effect on the amount of silica lost in the filtrate of the weight of silica present, 0.125 grm. quantities being used for this purpose.

*Commercially Pure Iron (99.84 per cent. of iron).*—The following results were obtained:

Weight of silica taken. Grm.	Weight of iron taken. Grms.	Ratio $\text{SiO}_2:\text{Fe}$ .	Weight of silica recovered from filtrate. Grm.
0.25 ..	0.08	1 : 0.32	0.0418
	0.15	1 : 0.6	0.0256
	0.25	1 : 1	0.0168
	0.38	1 : 1.52	0.0086
	0.50	1 : 2	0.0065
	1.00	1 : 4	0.0046
	1.50	1 : 6	0.0030
0.125	2.00	1 : 8	0.0010
	0.04	1 : 0.32	0.0276
	0.125	1 : 1	0.0112
	0.50	1 : 4	0.0014
	1.00	1 : 8	0.0002

Evidently the precipitation of silicic acid (probably as a ferric silicate) by addition of ammonium hydroxide to the filtrate from the first evaporation is complete, provided sufficient iron is present. This will be the case even in the higher grade ferro-silicons, as an experiment showed that the amount of silica passing into the filtrate after *one* evaporation was approximately 0.01 grm. For this purpose, 1 grm. of the silica and 0.5 grm. of iron were taken, as representing approximately a 50 per cent. ferro-silicon.

A further confirmation was obtained by fusing 1 grm. of silica and adding 0.5 grm. of iron as chloride (*i.e.* the approximate equivalent of a 1 grm. sample of 50 per cent. ferro-silicon), and taking it through the suggested method for the analysis of ferro-silicon outlined below. The 1 grm. of silica taken actually contained 0.9879 grm. of true silica, and 0.9878 grm. was recovered after the deduction of the blank.

**SUGGESTED METHOD OF FERRO-SILICON ANALYSIS.**—Fuse 1 grm. of the finely divided sample with 10 grms. of sodium carbonate and 5 grms. of sodium peroxide



well mixed in a nickel crucible. Extract the cake with hot water, transfer to a basin and, when completely disintegrated, cover the basin with a clock glass and run in an excess of concentrated hydrochloric acid from a pipette through the lip of the dish.

When effervescence has ceased, rinse the underside of the clock glass into the basin and evaporate the liquid to dryness on a water bath. Break up the residue with a glass rod and bake for 2 hours in an air oven at a temperature not exceeding 110° C. Moisten the residue with 20 c.c. of hydrochloric acid (sp. gr. 1.1), and warm for 5 minutes on the water bath. Add about 100 to 150 c.c. of hot water and filter immediately through a No. 40 Whatman paper, washing with hot 5 per cent. hydrochloric acid. Add excess of concentrated ammonia to the filtrate, boil and filter, washing twice with hot water. Make a hole in the paper and wash the precipitate into the beaker from which it was precipitated.

Extract the paper with hydrochloric acid and hot water, adding more hydrochloric acid to the solution in the beaker and warming until completely dissolved. Evaporate and dehydrate as above, then take up the residue with 10 c.c. of hydrochloric acid (sp. gr. 1.1) warm for a few moments, dilute with *cold* water and filter through a No. 44 Whatman paper, washing with *cold* 1 per cent. hydrochloric acid (about six times). Transfer both papers to a platinum crucible and ignite in a *muffle*, retaining the lid in position until all volatile matter has been driven off. Heat as usual.

In conclusion, I wish to thank Mr. H. V. Thompson for suggestions and criticism during the course of this work.

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## A New Method for Determining Traces of Chromium in Steel.\*

By W. J. AGNEW, B.A.

A METHOD for determining traces of chromium in steel has been described by Evans (ANALYST, 1921, 46, 38). Although giving good results, this method of pouring the oxidised chromium into excess of sodium hydroxide solution, followed by excess of acetic acid, has some disadvantages, notably when nickel and cobalt are present, requiring a further separation of these two elements with sodium hydroxide before proceeding with the colorimetric estimation. Evans (ANALYST, 1921, 46, 285) drew attention to the application of diphenylcarbazine, which gives

\* Communication from the Research Department, Woolwich.

a purple colour with dichromate. This reaction is very sensitive, 17 parts per million being detected, and  $N/1000$  potassium dichromate can be used as a standard.

An advantage of the method about to be described is that the determination can be made on a weight of 0.1 grm. of steel, if necessary. In most cases a weight of 1 grm. is a reasonably representative sample, from which, after dissolving, an aliquot portion is taken equal to 0.1 grm. of the original sample.

**METHOD.**—The method is as follows:—One grm. of the steel is dissolved in 15 c.c. of dilute sulphuric acid (1:3) and 20 c.c. of water and oxidised with 5 c.c. of nitric acid (sp. gr. 1.2).

The solution is boiled until free from nitrous fumes, cooled, and made up to a volume of 200 c.c. with distilled water. Forty c.c. of this solution, corresponding to 0.2 grm. of steel, are oxidised with 3 drops of saturated potassium permanganate solution, boiled for a few minutes, and the excess permanganate destroyed with a few drops of hydrochloric acid (sp. gr. 1.2), which is added, drop by drop, until the solution becomes quite clear; usually about 50 drops are required.

It is now immediately cooled, and the iron, etc., precipitated with a slight excess of sodium carbonate (saturated solution), diluted to 100 c.c. with distilled water, and filtered. Fifty c.c. of the filtrate, corresponding to 0.1 grm. of steel, are rinsed into a 600 c.c. flask, acidified with 20 c.c. of (1:3) sulphuric acid, 5 c.c. of a 0.1 per cent. solution of diphenylcarbazide added, and the whole transferred to a 100 c.c. Nessler glass. Into another Nessler glass are poured 20 c.c. of (1:3) sulphuric acid, 5 c.c. of the diphenylcarbazide solution, and about 70 c.c. of water, and the determination made by adding  $N/1000$  dichromate solution to the standard until the solutions are matched in tint.

**EXPERIMENTAL RESULTS.**—The following figures were obtained for electrolytic iron to which known amounts of chromium had been added.

#### ELECTROLYTIC IRON WITH CHROMIUM.

Iron taken.	$N/1000$ potassium dichromate added. c.c.	$N/1000$ potassium dichromate found on 1/10 vol. c.c. $\times 10$ .	Chromium added. Per Cent.	Chromium found. Per Cent.
1.0	1	0.1	0.0017	0.0017
1.0	2	0.2	0.0034	0.0034
1.0	4	0.4	0.0068	0.0068
1.0	10	1.0	0.017	0.017
1.0	20	2.0	0.034	0.034
1.0	25	2.5	0.042	0.042
1.0	30	3.0	0.051	0.051

The following figures were obtained for electrolytic iron containing various amounts of other elements.

## IRON WITH COPPER AND CHROMIUM.

Iron taken. Grm.	Copper added. Per Cent.	Chromium added. Per Cent.	Chromium found. Per Cent.
1.0	1.0	0.0017	0.0017
1.0	1.0	0.0034	0.0034
1.0	1.0	0.0051	0.0051
1.0	1.0	0.0068	0.0068
1.0	1.0	0.0085	0.0085
1.0	1.0	0.017	0.017
1.0	1.0	0.026	0.026
1.0	1.0	0.035	0.035
1.0	1.0	0.050	0.050

## IRON WITH NICKEL AND CHROMIUM.

Iron taken. Grm.	Nickel added. Per Cent.	Chromium added. Per Cent.	Chromium found. Per Cent.
1.0	15.0	0.0017	0.0017
1.0	15.0	0.0034	0.0034
1.0	15.0	0.0050	0.0050
1.0	7.5	0.068	0.068
1.0	7.5	0.034	0.034
1.0	7.5	0.087	0.085
1.0	7.5	0.087	0.087

## IRON WITH MOLYBDENUM AND CHROMIUM.

Iron taken. Grm.	Molybdenum added. Per Cent.	Chromium added. Per Cent.	Chromium found. Per Cent.
1.0	2.70	0.0085	0.0085
1.0	2.70	0.0170	0.0170
1.0	2.70	0.068	0.068

## IRON WITH VANADIUM AND CHROMIUM.

Iron taken. Grm.	Vanadium added. Per Cent.	Chromium added. Per Cent.	Chromium found. Per Cent.
1.0	1.0	0.0017	0.0017
1.0	1.0	0.0034	0.0034
1.0	1.0	0.034	0.033
1.0*	1.0	0.0034	0.0026

\* Colour in this case was a slightly different shade, due probably to vanadium.

## IRON WITH TUNGSTEN AND CHROMIUM.

Iron taken. Grm.	Tungsten added. Per Cent.	Chromium added. Per Cent.	Chromium found. Per Cent.
1.0	1.0	0.0017	0.0017
1.0	1.0	0.0034	0.0034
1.0	1.0	0.017	0.017
1.0	1.0	0.034	0.034

### IRON WITH COBALT AND CHROMIUM.

Iron taken. Grm.	Cobalt added. Per Cent.	Chromium added. Per Cent.	Chromium found. Per Cent.
1.0	2.0	0.034	0.034
1.0	2.0	0.0017	0.0017
1.0	2.0	0.0050	0.0050

### HIGH-CHROMIUM STEEL.

The following result was obtained on a steel containing a high percentage of chromium, compared with the percentage obtained by Evans's method:

Steel taken. Grm.	Chromium found. Per Cent.	Chromium found (Evans's method). Per Cent.
1.0	3.45	3.45

**INFLUENCE OF HYDROCHLORIC ACID.**—The following experiment was carried out to show that hydrochloric acid does not reduce the potassium dichromate under the conditions described. Each trial contained 2 c.c. of *N*/1000 potassium dichromate solution, to which was added 40 c.c. of distilled water, 3 c.c. of (1:3) sulphuric acid and 2 c.c. of nitric acid (sp. gr. 1.2); each was oxidised with potassium permanganate, and to A was added 30 drops of hydrochloric acid, to B 40 drops of hydrochloric acid, to C 50 drops of hydrochloric acid, and to D 100 drops of hydrochloric acid. They were then heated until clear, cooled, and diluted to 100 c.c. The colour in each case was the same on testing with diphenylcarbazine.

These results show that none of the dichromate was reduced, and as much as 15 per cent. of hydrochloric acid is permissible, provided the solution is not boiled too long.

**INFLUENCE OF TIME OF BOILING.**—The following experiments were carried out to show the influence of the time of boiling, after adding hydrochloric acid:

<i>N</i> /1000 potassium dichromate added. Drops.	Water added. c.c.	Hydrochloric acid added. Drops.	Time of boiling. Minutes.	Strength HCl. c.c. per 100 c.c.	<i>N</i> /1000 potassium dichromate found. Drops.
5	25	20	5	4.8	4
5	25	40	5	9.6	3
5	25	60	5	14.4	1
5	25	80	5	19.2	Trace
5	25	100	5	24.0	Nil
5	25	100	Just to boiling point	24.0	Nil
5	25	10	5	2.0	4
5	25	10	Just to boiling point	2.4	5

It will be observed from these results that prolonged boiling is fatal, since it leads to reduction of the potassium dichromate added.

**CONCLUSION.**—These test experiments show that chromium can be determined in steel by oxidising with potassium permanganate and reducing the excess of permanganate with hydrochloric acid, provided that the solution is cooled immediately the potassium permanganate is reduced; the experiments also prove that under those conditions the presence of as much as 15 per cent. of hydrochloric acid is permissible.

It should be mentioned that the chromium in all of these experiments was used in the form of potassium dichromate and was reduced before being added to the iron.

## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### AN APPARATUS FOR THE DETECTION OF TRACES OF FLUORIDE BY THE ETCHING METHOD.

FOR the detection of small amounts of fluoride by the etch test, it is necessary to concentrate the acid vapours in a small space, sealed so as to permit of prolonged action without fear of loss. These requirements are met in the following simple and easily manipulated piece of apparatus.

It consists of a casting of lead, 2 inches across the foot,  $1\frac{1}{2}$  inches across the mouth, and 2 inches high. The mouth is ground flat, and polished, and the cavity is about  $\frac{1}{4}$  inch in diameter and 1 inch deep, having a capacity, therefore, of the order of 3 to 4 c.c.

The manipulation is as follows: The fluoride is concentrated into a small bulk of inorganic material, by precipitation or ashing with the usual precautions to avoid loss, and transferred to the leaden "test-tube." A moderately thick  $\frac{3}{4}$ -inch coverslip, preferably square for convenience in later handling, is covered on one side with high-melting paraffin wax, and the glass bared, in the centre of the slide, in a small but distinctive design, by means of a sharp splinter of wood, or a bone stylus (not metal). The material in the tube is rapidly and thoroughly mixed with a few drops of concentrated sulphuric acid, and the prepared coverslip taken up with forceps in one hand, while a few rapid strokes are made over the mouth of the tube with a Bunsen flame held in the other. The coverslip is then at once placed on the tube, waxed side down, when, if the manipulation has been correctly carried out, the wax melts just where it touches the warm metal, without encroaching on the design, and rapidly re-solidifies, thus hermetically sealing the cavity. The whole apparatus can then be placed in an incubator at  $37^{\circ}$  C. for as long as desired.

When the incubation is complete, the coverslip is melted off, the wax removed with ether, and the glass examined with a powerful lens, by means of light reflected from the etched surface. Under these conditions, a faint etching will be revealed as a dark shadow against the background of reflected light.

The apparatus was devised some years ago for the detection of traces of fluoride in fruit pulp, and I have found it possible to detect, with certainty, 2 mgrms. of sodium fluoride added to 100 grms. of pulp, with overnight incubation. Other workers, to whom the apparatus has been described, report a similar degree of sensitiveness. In my experience the detection of smaller quantities of fluoride could not be relied upon, and it would seem that some specimens of coverslip glass are more sensitive to the action of hydrogen fluoride than others.

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### BLACK DRAUGHT.

*Mistura Sennae Composita*, B.P., is an article which has been subjected to change with every new edition of the *British Pharmacopoeia*. In the 1914 edition, the alterations in the composition of Compound Tincture of Cardamoms and Infusion of Senna (two of the ingredients) involved some difference in the resulting Black But Draught. In both the 1898 and the 1914 editions, magnesium sulphate was, and is, incorporated in the proportion of five parts by weight in twenty fluid parts of the finished article. That is, the proportion of magnesium sulphate is 25 per cent. w/v. This means that the theoretical proportion of anhydrous magnesium sulphate is 12·2 per cent.

In 1902, the Local Government Board for Ireland, in proposing a "Schedule of Standards for Pharmacopoeial Preparations," included one that the ash of this preparation should be 9·5 per cent. It may be that (in spite of a concomitant declaration in the "standards" that the percentage of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  was 25) the Local Government Board for Ireland had really based their standard, not on the 1898 *Pharmacopoeia*, but on that of 1885, where a smaller content of Epsom salts was directed. In any case, the ash figures detailed below show the results obtained on samples bought in four different counties during 1930. The ash figures were the results of igniting the total solids of five c.c. in porcelain milk dishes. The last sample is obviously not satisfactory.

Specific gravity at 15·5°C.	Total solids per cent. (w./v.).	Ash per cent. (w./v.).
1·1216	21·63	11·23
1·1170	20·64	11·40
1·1118	19·62	11·44
1·1313	24·20	11·46
1·1146	22·02	11·52
1·1208	21·12	11·60
1·1183	21·68	12·08
1·1291	20·88	12·16
1·1283	20·52	12·62
1·1268	22·26	12·68
1·1375	23·50	14·20

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### HOLDER FOR MERCURIC CHLORIDE PAPER IN GUTZEIT TEST.

SUGGESTIONS for holding the mercuric chloride paper have appeared in the journal from time to time (ANALYST, 1927, 52, 699, 700, 701; 1928, 53, 152; 1930, 55, 503, 630), but the apparatus described below has the advantage of being easily and cheaply constructed, and of producing uniform stains with well-defined edges.

A tin screw cap arrangement is cut from a "Cardboard Screw Cap Post Box," and forced over a tightly fitting rubber bung. The bung has one hole in the centre, 8 mm. in diameter, and the tin screw cap also has a hole drilled through its centre, 8 mm. in diameter. The mercuric chloride paper is placed on the rubber bung covering the hole, the cap is screwed on, and the bung pushed on the end of the Gutzeit tube, so that the gas must pass through the paper. This apparatus, has been found efficient in this laboratory for some years. Its size is not important, so long as the 8 mm. holes in the rubber bung and the tin cap coincide.

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## Notes from the Reports of Public Analysts.

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF BIRMINGHAM.

#### REPORT OF THE CITY ANALYST FOR THE THIRD QUARTER, 1929.

THE number of samples analysed during the quarter was 1198, of which 51 were formal samples; of the total number 42 were incorrect.

CREAM.—Two samples (one formal, the other informal) from the same vendor were found to contain 0.12 and 0.15 per cent., respectively, of boric acid. A summons was taken out under the Food and Drugs Act, since no penalty is provided under the Preservative Regulations unless it is proved that the offence was wilfully committed. A fine of 10s. was imposed.

H. H. BAGNALL.

### METROPOLITAN BOROUGH OF STEPNEY.

#### ANNUAL REPORT OF THE BOROUGH ANALYST FOR THE YEAR 1929.

OF the 1609 samples examined, 1537 were purchased under the Food and Drugs Act, 951 being formal (49 adulterated), and 586 informal (14 adulterated).

MILK.—One of the 923 samples examined was coloured with annatto. Formaldehyde (3 parts per million) was present in two samples taken during the hot weather. The vendor was fined £2 with £3 3s. costs.

ICE CREAM.—Four samples examined were genuine. Three samples contained in each case 2.6 per cent. of milk fat and were thickened with starch. One

sample contained 7.1 per cent. of fat and was free from starch filling. There is no legal standard controlling the composition of ice cream.

**TABLE JELLY.**—One sample (informal) containing 90 parts of sulphur dioxide per million was labelled in a misleading and unsatisfactory manner. An article of food may contain preservative if the constituents from which it is made are allowed to contain preservative. These constituents must not, however, in the final product, contain more than the maximum amount of preservative allowed in each constituent by the Regulations.

The Medical Officer of Health informed the retailers that the jelly contained preservative within the permitted amount, but that the notice created an impression that the jelly did not contain preservative, and that the notice should be withdrawn or altered in such a way as not to create a wrong impression. The retailers finally stated that the notice on the carton would be deleted in future (*cf.* ANALYST, 1930, 55, 279).

**CARBOLIC POWDER.**—Eleven samples were examined for the Borough Engineer. Of these, 10 complied with the requirements of the specification, and one was unsatisfactory. The specification states that "carbolic powder must contain not less than 15 per cent. of tar acids calculated as cresylic acid, the base to consist of siliceous or other inert matter." During the period that these examinations have been made, the following unsatisfactory powders have been condemned: (1) Chalk containing 4 per cent. of tar acids; (2) flue dust mixed with wood tar creosote containing 9 per cent. of acidic oils of doubtful disinfectant value; and (3) spent gas lime containing 9 per cent. of naphthalene.

DOUGLAS HENVILLE.

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### WARRANTIES AS TO CATTLE CAKE.

G. C. DOBELL & Co., LTD., *v.* BARBER AND GARRATT.

THIS was an appeal from the judgment of Mr. Justice Roche (ANALYST, 1930, 55, 447), who had held that the plaintiffs, who were merchants, were entitled to a declaration on the question of warranty, but were not entitled to recover from the defendants the damages which they had had to pay to their buyers for injury caused to stock through the cattle cake containing castor seed. The plaintiffs appealed on the question of the measure of damages, and the defendants appealed on the question of liability.

Lord Justice Scrutton, in his judgment, agreed with Mr. Justice Roche that there was a sale by the defendants and a purchase by the plaintiffs of linseed cake, within the meaning of Sec. 2 (2) of the Act of 1926; and that the warranty of suitability was not excluded by Sec. 24. The defendants' cross-appeal must, therefore, be dismissed with costs.



On the question of the measure of damages, the plaintiffs were entitled to rely upon a statutory warranty, and they had statutory authority for disregarding any notice or contract to the contrary, and an analysis by a responsible firm that the cake was "castor free." The defendants said that they accepted no responsibility for the analysis. It was their only way of complying with the statutory obligation to give a correct statement as to the oil and albuminoids in the cake. Parliament had intended the vendors of cattle food to take responsibility for its fitness for the purpose, and if the vendor wished to escape his responsibility he should not sell food only used for that purpose. The appeal should be allowed, and the judgment by Mr. Justice Roche varied by declaring that the plaintiffs were entitled to include in their damages the damages and costs reasonably incurred in claims by their sub-purchases for breach of their statutory warranty.

Lord Justice Lawrence concurred.

Lord Justice Greer dissented. He held that Sec. 24 of the Act of 1926, excluded the operation of that Act in the circumstances of this case. He was not prepared to hold that Mr. Justice Roche was wrong.

#### REMOVAL OF MARKS FROM EGGS WITH ACETIC ACID.

ON December 3rd, a shopkeeper was summoned, at the Lavern Petty Sessions (East Yorkshire), for obliterating marks of origin from imported eggs in order to sell them as English.

An inspector under the Merchandise Marks Act gave evidence that he had visited a warehouse of the defendant in Hornsea. In one box he saw eggs bearing the mark "Holland," and in two other boxes damp eggs showing signs that the identification marks had been rubbed off. Samples of the eggs were taken and sealed in bottles for analysis, and a sample of liquid was also taken from a jar.

The Public Analyst's certificate showed that the eggs had been treated to remove the marks of origin, and that the liquid from the jar was strong acetic acid.

The Bench convicted and imposed a fine of £5.

## Queensland.

### REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR ENDING JUNE 30, 1930.

In his annual report Dr. J. B. Henderson states that 7947 samples were examined during the year, 2748 of which were for the Health Department. Of the total samples of foods and drugs submitted, 1511 were legal samples taken by the inspectors, in accordance with the provisions of the Health Act, and, of these, 305 were adulterated or not up to standard.

**LEAD ARSENATE IN CABBAGE.**—Ten samples of cabbage contained lead arsenate in quantities ranging from 3.2 to 9.3 grains per cabbage, showing that this dangerous menace is not yet removed.

**SULPHUR DIOXIDE IN FRUIT AND MINCED MEAT.**—Eight samples of preserved fruit contained sulphur dioxide at the rate of 8 to 18·9 grains per pound, whereas the maximum amount permitted to be present under the Food and Drug Regulations is 7 grains.

The use of sulphur dioxide is prohibited in minced meat, but eleven samples of minced meat received contained sulphur dioxide at the rate of 1·9 to 14·4 grains per pound. With cold storage so easily and cheaply available there is no excuse for adding preservatives to minced meat.

**ORANGE BEVERAGES.**—Out of thirty samples of orange beverages examined, 18 contained less than 3 per cent. of orange juice. It is important, from the standpoint of national health, as well as from that of the fruit-growing industry, that the minimum proportion of orange juice, namely, 5 per cent., required in an orange beverage, should be maintained.

**POLICE DEPARTMENT.**—For the Police Department, 208 samples were analysed, a distinct increase on the work of the previous year. In connection with 47 cases of death from suspected poisoning, 61 samples of viscera and 78 other samples of poisons, medicines, foods, etc., were examined. In 17 cases no poison was found, but in the other 30 cases poison was found as follows:—Strychnine, 21 cases; lysol, 2 cases; cyanide, 2 cases; carbon monoxide, sodium fluoride, arsenic, zinc chloride, and caustic soda, 1 case each.

Strychnine has of late years been gradually becoming more prominent in these cases, mostly suicides, and in 1929 was more than two-thirds of the total, probably because strychnine is now the most readily obtainable poison.

Sodium fluoride, which is used as a cockroach powder, provided its first recorded fatal case in Queensland. In connection with a case of attempted suicide, five exhibits were examined and strychnine found present. In 4 cases of alleged attempted food poisoning, 24 samples were submitted; strychnine was found in 2 samples, and cresol in 3 samples.

In 11 cases of suspected animal poisoning, 17 samples were examined, comprising viscera, opossum baits, dog baits, and water. In three cases poison was found, cyanide 1 case, strychnine 1 case, and arsenic 1 case; in the other eight cases no poison was found.

## Government of Palestine.

### ANNUAL REPORT OF THE DEPARTMENT OF HEALTH (CHEMICAL DIVISION) FOR THE YEAR 1929.

THE number of samples analysed during the year under the direction of the Government Analyst (Mr. G. W. Baker) was 6388, and much advisory work was undertaken for various departments, especially the Department of Customs, Excise and Trade.

**MILK.**—The control over milk supplies is of necessity decentralised, the analysis being carried out in 13 sub-districts with Gerber outfits; weekly returns from all sub-districts are scrutinised at the Central Laboratories. The question of equitable standards of composition is a difficult one, as some of the milk comes from well-kept herds of imported cattle, while much is a mixture of milk from wandering

herds of the small local cow and the goat. On the rather low standard of a minimum of 3 per cent. of fat and 8 per cent. of other solids, prosecutions were instituted in 3.7 per cent. of the 3892 samples taken.

**EDIBLE OILS.**—The analysis of 182 samples of edible fats and oils showed 18 per cent. of these to be adulterated. It has been found necessary to fix a limit to the acidity of edible olive oil supplied by contract to prisons. Provisionally, this maximum limit has been fixed at 15 per cent. This is, perhaps, somewhat high, but the local palate prefers a strong flavour. It might here be mentioned that the Customs tariff admits unrefined olive oil into Palestine free of duty, on the understanding that it is for soap-making, but there is little to prevent its being sold as edible oil to the prejudice of the consumer and the local oil producers.

**LEGAL, JUDICIAL AND POLICE DEPARTMENTS.**—The work undertaken for these Departments has involved the examination of 209 specimens and court exhibits, as compared with 164 last year.

**Counterfeit Coins.**—Of 78 coins submitted, 77 have been confirmed to be counterfeit. The outstanding features of most of these coins have been the same as those noted in last year's report, with the exception of two well-made 50 mil pieces containing approximately 57 per cent. silver. These coins are evidently struck and not cast, and are the first examples of struck counterfeit new currency to come under official notice. In one case in Haifa a large number of 100 mil pieces of pure tin were discovered by the police, together with iron moulds and sticks of pure tin.

**Oil Stains.**—In a case of burglary, where an iron bar on a window had been cut, a bottle containing a mixture of mineral oil and olive oil had been left on the window sill. Stains were noticed on the jacket of a suspect, and, on examination of these stains, the presence of freshly-cut fragments of iron and traces of oil of the same character as that in the bottle was discovered.

**Firearms.**—Much time has been spent upon the examination of firearms and ammunition in connection with murders committed during the disturbances. The laboratory investigation resulted in conclusive evidence that a cartridge case found on the premises where five persons were murdered had been fired in the rifle in possession of the accused person. As this case presents many interesting features from the scientific point of view it was decided to prepare a detailed account for publication elsewhere. (*Cf. ANALYST, 1930, 55, 738.*)

**DEPARTMENT OF CUSTOMS, EXCISE AND TRADE.**—A total of 1572 samples has been examined for this Department, compared with 1291 last year.

**Acid Oils for Soap-making.**—If these contain not less than 30 per cent. free acidity (calculated as oleic acid) they are now exempt from import duty. This has necessitated the examination of samples from 277 consignments of such oils.

**RAILWAYS AND HAIFA HARBOUR DEPARTMENT.**—In the experience of the railways, boiler tubes are liable to be perforated after a few months' use with Haifa water. Magnesium chloride is apparently the cause of most of the corrosion, and in some of the wells this constituent (calculated as a "probable combination") amounts to as much as 35 parts per 100,000. Added to this there is a sodium chloride content high enough to give serious trouble.

## The Composition of Fruits as used for Jam Manufacture in Great Britain.

A COMMUNICATION FROM THE BRITISH ASSOCIATION OF RESEARCH FOR THE COCOA, CHOCOLATE, SUGAR, CONFECTIONERY AND JAM TRADES.—T. MACARA, F.I.C., *Director of Research*.

DURING the past two years a joint committee of Public Analysts and representatives of the Jam Section of the Food Manufacturers' Federation have been engaged in discussing the question of Standards for Jams. Eventually agreement was reached, and a schedule of Standards and definitions was drawn up, copies of which were circulated to all members of the Society of Public Analysts and other Analytical Chemists (see ANALYST, 1930, 55, 694). These, of course, have at present no legal standing, as they have not been issued by a Government Department. In the absence of any such legal standards, however, analysts and manufacturers alike felt that the time had come when standards for jams were necessary, in the interests of both the public and the manufacturers themselves.

In order to assist analysts in the exceedingly difficult task of determining the fruit content of jams, the Council of the British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades willingly consented to the publication of the results of a large number of analyses of jam fruits which its staff has made during the past seven years. This paper deals, therefore, with these analyses, describing the methods used and giving a summary of the results in detail in tables. Finally, suggestions are made as to how the figures given in the tables may be used in determining the fruit content of a jam.

**THE SAMPLES OF FRUIT.**—The samples of fruits were spread over a number of seasons, and were of varying origin, with a view to establishing not merely average figures for the composition of each kind of fruit, but also the extent of the variation likely to occur in those constituents which are of analytical interest. A certain proportion of Scottish fruits and fruits of foreign origin has been included. It should be emphasised that the selection could not be anything but an arbitrary one, so that in a sense the use of the word "average" is misleading—in fact, it does not seem possible to define anything that can be regarded as a true "average" composition of a fruit. The extreme figures, however, rest on a surer basis; they have been obtained once, and may be obtained again, or even exceeded. It is always possible that they may be extended in either direction by samples from fresh districts, by fresh varieties, or by the effects of an unusual season. In using them, discretion should be applied, in regard to the likely effect of condition of fruit, seasonal conditions, and so on.

Only those proximate constituents of the fruits which were of interest to the manufacturer or the analyst were determined. These were soluble solids, insoluble matter, pectin, sugars, and acidity. In some cases certain other features of interest were examined, mostly of a non-chemical nature; *e.g.* proportion of seeds in raspberries, etc.

### ANALYTICAL DETAILS.

About 1 lb. of fruit, taken from a larger mixed sample, was passed through a mincer (care being taken to avoid any loss of juice), and 100 or 150 grms. of the well-mixed mass were weighed out, and boiled moderately for an hour with about 400 or 600 ml. respectively, of water in a large beaker, with occasional stirring and addition of water to maintain the volume. After cooling to room temperature, the mixture was transferred to a measuring cylinder, made up to 500 or 750 ml.

with water, and the whole thoroughly mixed by vigorous shaking. The extract was then strained off through a 120 mesh sieve or filtered through a large fluted paper, of a texture which permitted fairly rapid filtration. In some cases, for example, apples, it was necessary to interrupt the boiling after twenty minutes or half-an-hour, to rub the mixture through a coarse sieve, and then resume the boiling; this broke up lumps which otherwise would not have been thoroughly extracted. With cherries, a definite weight of whole fruit was boiled, and the stones were removed from the mixture before making up to volume. The same procedure was adopted for some of the plums; with others, it was possible to detach the stones beforehand, so that the analysis could be carried out on the minced flesh only, from the outset. The results were calculated back to 100 parts of the stoneless fruits, whether the stones were removed before or after boiling.

**SOLUBLE SOLIDS.**—The specific gravity and refractive index (by immersion refractometer) of this extract were determined, and from these figures the percentage of soluble solids was calculated by means of the ordinary sugar factors. The figures given in the tables are the means of the gravity and refractometric results.

**SUGARS.**—A suitable quantity of the extract was treated with lead subacetate, made to volume, filtered, and the excess of lead in a portion of the filtrate was removed with sodium phosphate. The final filtrate was then inverted at 60° C. with hydrochloric acid, cooled and neutralised with sodium hydroxide solution, and the reducing sugar was determined by copper reduction, either by the gravimetric method of Quisumbing and Thomas (in the earlier seasons) or by the volumetric method of Lane and Eynon. The results for total sugars may be considered to correspond to a sugar mixture more or less approximating to invert sugar in composition.

**ACIDITY.**—A quantity of extract, usually 50 ml., was diluted with several hundred ml. of distilled water and titrated with decinormal sodium hydroxide to the appearance of a colour with phenolphthalein. A blank titration of the distilled water was made at the same time, and deducted. The acidity is for convenience expressed as crystallised citric acid, except for apples, where it is given as malic acid (a figure not very different).

**PECTIN.**—In the earlier years this was determined in the extract (filtered through paper if only strained previously) by the method of Carré and Haynes (*Biochem. J.*, 16, 63). In the later seasons it was found possible to handle a larger quantity of precipitate (up to 0.1 grm.) than was recommended by these workers. Consequently, the usual procedure adopted was to treat 50 c.c. of the 20 per cent. extract as described by Carré and Haynes, except that the time of saponification was shortened to 2 hours by keeping the volume of extract and alkali at about 100 c.c. After saponification this was diluted to 400 c.c. before precipitation of the pectate, which was afterwards filtered off on tared filter papers. The results are expressed as percentages of crude calcium pectate. A number of analyses were made in 1927, when the crude pectin was precipitated first by acetone and the true pectin determined in this precipitate. The percentage obtained in this way was generally within 0.1 per cent. of that obtained as crude pectate, except in a few fruits where the pectate was over 1 per cent. As it appeared that little was to be gained by elaborating the process in this manner, the work was continued by means of the direct process, which should, therefore, be used in the analysis of jams.

**INSOLUBLE MATTER.**—Ten or twenty grms. of the well-mixed minced material were weighed off, diluted with 100 to 200 ml. of distilled water, and boiled for half-an-hour. The mixture was poured on to a tared filter, the residue returned to the beaker and again boiled with a further quantity of water. The insoluble

matter was finally washed on the filter with plenty of boiling water and dried in the oven at 105° C. to constant weight.

**REMARKS.**—The figures obtained for the extracts were calculated back to percentage of the whole fruit except in the case of stone fruit, allowance being made for the effect of the volume of insoluble matter in causing the extracts to be not quite true "20 per cent." extracts. In most cases this effect was very small. The determinations were carried out on duplicate 20 per cent. extracts, and on duplicate portions for the insoluble matter.

The results, which are the mean of duplicates, have been expressed to the nearest 0.05 per cent. in the case of insoluble and soluble solids and sugars, as this represents about the probable limit of accuracy in these determinations.

Gooseberries and raspberries were analysed as received, but strawberries were "plugged" and currants had the "strigs" removed. Apples were analysed whole, *i.e.* were not peeled or cored.

#### VARIATIONS IN COMPOSITION.

The individual data have been summarised in the first Table, which shows the highest and lowest values found for each analytical figure, together with the averages (arithmetic means) for each type of fruit.

In order to afford a clearer view of the actual significance of the average figures, an analysis of some of the data has been made (Tables II to IV), so as to display the numbers of samples falling within definite ranges of values. In most cases, the average figure lies within the most frequently occurring range. The insoluble solids content of gooseberries, strawberries, and raspberries, however, occurs in greater frequency in a range a little below the average figure. The same applies to the acidity of Victoria plums, and to the pectin content of blackcurrants, Victoria plums, and damsons. The acidity of the cherries seems to fall into two groups, with the average figure dividing them.

The presentation of the data in this way may be useful also in showing the likelihood of occurrence of the extreme cases shown in Table I, some of which were undoubtedly abnormal (*e.g.* the very high figure of 1.31 per cent. pectin in apples; the high acid figure of 3.62 per cent. for damsons; and the high insoluble solids of 4.55 per cent. in gooseberries, and 5.95 per cent. in apples).

#### USE OF THE DATA IN THE INTERPRETATION OF JAM ANALYSES.

It should be emphasised that if it is desired to use the present data in interpreting the results of a jam analysis, the jam should be analysed in a manner similar to that adopted here for the fruits themselves. Thus, in making an extract of the jam, the sample should be boiled with a sufficient amount of water for about an hour. This is particularly important in regard to the pectin estimation in jams from fruits containing rather tough fibrous matter such as blackcurrants or blackberries.

In determining the fibre, pectin and acid in a jam, it is essential to use a proportion of water to jam, which is not less than that used in the analyses of the fruits, and to boil for a similar period. That is, the quantity of water used should be at least four times that of the sample, and the mixture should be boiled for about one hour, the volume of solution being maintained reasonably constant.

In sampling the jam the greatest care should be taken to see that the whole contents of the jar are thoroughly mixed as the fruit fibre invariably tends to float to the upper layers. The attention of Food and Drugs Inspectors, who are responsible for the division of samples, should be directed particularly to this point.

The portion used for analysis should be either ground in a mortar (soft fruit jams) or put through a small mincer (jams made with tough skinned fruits). *Stones*, *e.g.* those of plums, etc., should, of course, be removed and their proportion noted, though as a rule no conclusions can be drawn from the percentage found, as manufacturers frequently remove a considerable number before filling the jam into the jars.

## INTERPRETATION OF THE ANALYSIS.

Generally the figures made use of in calculating the proportion of fruit in the jam are those for insoluble solids, acidity and pectin, all of which vary rather widely in different samples of the same fruit and render the exact determination of the fruit content almost an impossibility. Nevertheless it has been found possible, in the majority of cases of single fruit jams at least, to arrive at a fairly good approximation to the truth by a consideration of all three figures. Experience in the analysis of jams naturally is of great assistance, just as it is in the case of oils and fats.

It is suggested that in order to determine whether or not a jam is of a particular standard, the composition should first be calculated on the assumption that the fruit is of average composition. (See Table I.) Should the jam then fall below the standard it is advisable to use the three minima in this calculation. Should the jam still fall below the limits there can be little doubt that it has not been made with the standard weight of fruit and a certificate should be issued accordingly. Where it passes on this basis but does not come up to standard when using the average figures, it would be best to test further samples of the same make, and if all fall below the standard the manufacturer should be asked for an explanation.

Where the analysis is carried out on one sample only there are possibilities that a wrong conclusion may be drawn, for it is exceedingly difficult to ensure that the whole of the fruit in a boiling of jam will be evenly distributed throughout all the jars filled. When more than one jar is tested it will be found that while the insoluble solids may vary somewhat widely, the acid and pectin remain much the same in each. This is especially true of the acid, though the pectin may vary to a slight extent with the proportion of fibre. The acid figure will generally give a fair indication of the composition of the jam, except in those cases where acid has been added. A determination of the pH value of the jam will frequently indicate any marked addition of this sort, if compared with figures given by genuine full fruit standard jams.

## MIXED JAMS.

In the case of mixed jams the problem is naturally more complicated, and here it is useful to count the seeds, as in raspberry and gooseberry (see Table No. V), and, of course, a microscopic examination should also be carried out, comparing if possible with a jam of known composition or a known mixture of the fruits. In this way it has been found possible to detect adulteration with the seeds of raspberry in a raspberry and gooseberry jam. In one such case the quantity of raspberry was estimated to be about 10 per cent., although the seeds would have indicated nearer 40 per cent. The manufacturer afterwards stated that 12 per cent. had been used.

Mixtures of plums and apples are among the most difficult to deal with, but here a microscopic together with a macroscopic examination of the jam should help considerably.

## OTHER TESTS.

While it is recognised that the above methods of determining the fruit content of a jam leave much to be desired, the analyst will, as the result of his experience in analysing jams of undoubted standard quality, gain confidence in the results, but further methods of arriving at the fruit content are desirable. This fact has not been overlooked, and the staff of the Research Association has been engaged during the past fruit season in trying several new methods. Among these is one originally suggested by Mr. L. K. Boseley, which makes use of what is called the lead number. As originally described by Boseley the method did not give concordant results in all cases, but it has now been modified and developed as a routine method, and has already proved very helpful in many cases. When a sufficient number of figures for the various fruits have been collected, the method will be published.

Another figure which is of use in conjunction with others is the pH value, but here again a larger number of results is required before this can be applied with confidence to the estimation of the fruit content in jams.

TABLE I.  
EXTREME AND AVERAGE COMPOSITIONS OF FRUITS.

	Insol. solids (fibre, etc.). Per Cent.	Soluble solids. Per Cent.	Total solids. Per Cent.	Total sugars. Per Cent.	Acid as crystallised citric. Per Cent.	Pectin as crude cal- cium pectate. Per Cent.
<b>GOOSEBERRIES.</b>						
Highest .. ..	4.55	11.35	15.25	7.1	3.00	1.19
Lowest .. ..	1.7	6.9	9.1	2.0	1.47	0.50
Average (86 samples) ..	2.61	8.45	11.06	3.51	2.22	0.81*
<b>STRAWBERRIES.</b>						
Highest .. ..	3.45	13.6	16.2	8.5	1.74	0.78
Lowest .. ..	1.3	5.4	7.3	3.2	0.46	0.36†
Average (47 samples) ..	2.14	8.98	11.12	5.48	0.93	0.53†
<b>RASPBERRIES.</b>						
Highest .. ..	9.2	11.9	20.65	7.85	2.68	0.87
Lowest .. ..	4.4	5.4	10.9	1.3	1.23	0.37‡
Average (54 samples) ..	6.17	7.98	14.15	3.58	1.73	0.53‡
<b>REDCURRANTS.</b>						
Highest .. ..	7.6	12.65	19.7	6.9	2.95	0.67
Lowest .. ..	4.05	9.1	13.75	4.05	2.16	0.44
Average (9 samples) ..	6.02	10.17	16.19	4.80	2.54	0.58
<b>BLACKCURRANTS.</b>						
Highest .. ..	7.9	16.7	22.4	8.25	4.32	1.67
Lowest .. ..	4.7	10.0	17.25	2.25	2.70	0.63
Average (20 samples) ..	5.69	14.25	19.94	6.44	3.48	1.08
<b>CHERRIES (on stone-free fruit).</b>						
Highest .. ..	2.7	14.75	17.45	10.6	1.65	0.40
Lowest .. ..	0.95	10.7	12.35	6.9	0.41	0.11
Average (12 samples) ..	1.88	12.41	14.29	8.33	0.88	0.24
<b>VICTORIA PLUMS (on stone-free fruit).</b>						
Highest .. ..	1.6	15.2	16.65	9.1	2.19	1.07
Lowest .. ..	0.9	9.6	10.5	5.9	1.15	0.61
Average (14 samples) ..	1.13	12.63	13.76	7.43	1.64	0.81
<b>GREEN &amp; GOLDEN PLUMS (on stone-free fruit).</b>						
Highest .. ..	1.35	11.8	12.7	6.5	1.81	1.02
Lowest .. ..	0.85	9.1	9.95	4.5	0.97	0.67
Average (5 samples) ..	1.03	10.80	11.83	5.69	1.47	0.80
<b>RED &amp; MISCELLANEOUS PLUMS (on stone-free fruit).</b>						
Highest .. ..	1.7	17.05	18.35	10.25	2.79	1.21
Lowest .. ..	0.75	9.35	10.35	3.95	0.54	0.54
Average (15 samples) ..	1.22	13.10	14.32	7.56	1.74	0.82
<b>GREENGAGES (on stone-free fruit).</b>						
Highest .. ..	1.35	17.25	18.3	11.3	1.44	1.03
Lowest .. ..	0.95	10.6	11.75	5.35	1.04	0.86
Average (5 samples) ..	1.16	14.05	15.21	8.00	1.20	0.95
<b>APPLES.</b>						
Highest .. ..	5.95	13.55	18.35	9.75	1.84§	1.31
Lowest .. ..	1.6	9.5	12.15	4.2	0.52§	0.49
Average (28 samples) ..	2.57	11.70	14.27	7.60	1.11§	0.75
<b>DAMSONS (on stone-free fruit).</b>						
Highest .. ..	2.8	22.65	24.95	11.45	3.62	1.52
Lowest .. ..	1.25	10.55	12.75	3.9	1.80	0.95
Average (18 samples) ..	1.96	16.03	17.99	7.53	2.48	1.15
<b>BLACKBERRIES.</b>						
Highest .. ..	13.55	10.4	23.0	6.7	1.24	0.85
Lowest .. ..	6.6	7.85	14.45	3.3	0.52	0.22
Average (11 samples) ..	9.64	9.06	18.70	5.10	0.85	0.59

\* 63 samples.

‡ Excluding 10 sulphited samples.

† Excluding 3 sulphited samples.

§ As malic acid.



TABLE II.

## SPREAD OF VALUES FOR INSOLUBLE SOLIDS CONTENT.

## GOOSEBERRIES.

Range. Per Cent.	No. of samples.
1.55-2.0	7
2.05-2.5	36
2.55-3.0	28
3.05-3.5	12
3.55-4.0	2
4.05-4.5	0
4.55-5.0	1

Average 2.61 per cent.

## STRAWBERRIES.

Range. Per Cent.	No. of samples.
1.05-1.5	3
1.55-2.0	18
2.05-2.5	17
2.55-3.0	7
3.05-3.5	2

Average 2.14 per cent.

## RASPBERRIES.

Range. Per Cent.	No. of samples.
4.05-4.5	2
4.55-5.0	6
5.05-5.5	9
5.55-6.0	14
6.05-6.5	7
6.55-7.0	5
7.05-7.5	2
7.55-8.0	3
8.05-8.5	3
8.55-9.0	2
9.05-9.5	1

Average 6.17 per cent.

## REDCURRANTS.

Range. Per Cent.	No. of samples.
4.05-4.5	
4.55-5.0	
5.05-5.5	
5.55-6.0	
6.05-6.5	
6.55-7.0	
7.05-7.5	
7.55-8.0	1

Average 6.02 per cent.

## BLACKCURRANTS.

Range. Per Cent.	No. of samples.
4.55-5.0	5
5.05-5.5	5
5.55-6.0	5
6.05-6.5	3
6.55-7.0	0
7.05-7.5	1
7.55-8.0	1

Average 5.69 per cent.

## CHERRIES.

Range. Per Cent.	No. of samples.
0.55-1.0	1
1.05-1.5	2
1.55-2.0	4
2.05-2.5	2
2.55-3.0	3

Average 1.88 per cent.

## VICTORIA PLUMS.

Range. Per Cent.	No. of samples.
0.55-1.0	5
1.05-1.5	8
1.55-2.0	1

Average 1.13 per cent.

GREEN & GOLDEN  
PLUMS.

Range. Per Cent.	No. of samples.
0.5-1.0	3
1.05-1.5	2

Average 1.03 per cent.

## RED &amp; BLUE PLUMS.

Range. Per Cent.	No. of samples.
0.55-1.0	2
1.05-1.5	12
1.55-2.0	1

Average 1.22 per cent.

## GREENGAGES.

Range. Per Cent.	No. of samples.
0.55-1.0	1
1.05-1.5	4

Average 1.16 per cent.

## APPLES.

Range. Per Cent.	No. of samples.
1.55-2.0	7
2.05-2.5	8
2.55-3.0	9
3.05-3.5	3
3.55-4.0	0
4.05-4.5	0
4.55-5.0	0
5.05-5.5	0
5.55-6.0	1

Average 2.57 per cent.

## DAMSONS.

Range. Per Cent.	No. of samples.
1.05-1.5	3
1.55-2.0	8
2.05-2.5	5
2.55-3.0	2

Average 1.96 per cent.

## BLACKBERRIES.

Range. Per Cent.	No. of samples.
6.05-7.0	2
7.05-8.0	1
8.05-9.0	0
9.05-10.0	3
10.05-11.0	3
11.05-12.0	0
12.05-13.0	1
13.05-14.0	1

Average 9.64 per cent.

TABLE III.  
SPREAD OF VALUES FOR ACID CONTENT.

GOOSEBERRIES.		REDCURRANTS.		VICTORIA PLUMS.		GREENGAGES.	
Range. Per Cent.	No. of samples.	Range. Per Cent.	No. of samples.	Range. Per Cent.	No. of samples.	Range. Per Cent.	No. of samples.
1.41-1.60	1	2.01-2.20	1	1.01-1.20	1	1.01-1.20	3
1.61-1.80	6	2.21-2.40	1	1.21-1.40	1	1.21-1.40	1
1.81-2.00	14	2.41-2.60	4	1.41-1.60	7	1.41-1.60	1
2.01-2.20	19	2.61-2.80	2	1.61-1.80	2		
2.21-2.40	24	2.81-3.00	1	1.81-2.00	0	Average 1.20 per cent.	
2.41-2.60	11	Average 2.54 per cent.		2.01-2.20	3	APPLES.	
2.61-2.80	7			Average 1.64 per cent.		Range.	No. of
2.81-3.00	2					Per Cent.	samples.
Average 2.22 per cent.						0.41-0.60	2
STRAWBERRIES.		BLACKCURRANTS.		GREEN & GOLDEN PLUMS.		0.61-0.80	4
Range. Per Cent.	No. of samples.	Range. Per Cent.	No. of samples.	Range. Per Cent.	No. of samples.	0.81-1.00	6
0.41-0.60	2	2.61-2.80	1	0.81-1.00	1	1.01-1.20	7
0.61-0.80	13	2.81-3.00	3	1.01-1.20	0	1.21-1.40	4
0.81-1.00	15	3.01-3.20	2	1.21-1.40	1	1.41-1.60	2
1.01-1.20	13	3.21-3.40	2	1.41-1.60	1	1.61-1.80	2
1.21-1.40	3	3.41-3.60	3	1.61-1.80	1	1.81-2.00	1
1.41-1.60	0	3.61-3.80	3	1.81-2.00	1	Average 1.11 per cent.	
1.61-1.80	1	3.81-4.00	5	Average 1.47 per cent.		DAMSONS.	
Average 0.93 per cent.		4.01-4.20	0			Range.	No. of
RASPBERRIES.		4.21-4.40	1			Per Cent.	samples.
Range. Per Cent.	No. of samples.	Average 3.48 per cent.		RED & BLUE PLUMS.		1.61-1.80	1
1.21-1.40	10			Range.	No. of	1.81-2.00	1
1.41-1.60	11			Per Cent.	samples.	2.01-2.20	1
1.61-1.80	12			0.41-0.60	1	2.21-2.40	5
1.81-2.00	10			0.61-0.80	0	2.41-2.60	6
2.01-2.20	5			0.81-1.00	0	2.61-2.80	1
2.21-2.40	5			1.01-1.20	1	2.81-3.00	1
2.41-2.60	0			1.21-1.40	2	3.01-3.20	1
2.61-2.80	1			1.41-1.60	0	3.21-3.40	0
Average 1.73 per cent.				1.61-1.80	5	3.41-3.60	0
RASPBERRIES.				1.81-2.00	2	3.61-3.80	1
Range. Per Cent.	No. of samples.			2.01-2.20	1	Average 2.48 per cent.	
1.21-1.40	10			2.21-2.40	1	BLACKBERRIES.	
1.41-1.60	11			2.41-2.60	1	Range.	No. of
1.61-1.80	12			2.61-2.80	1	Per Cent.	samples.
1.81-2.00	10					0.41-0.60	2
2.01-2.20	5					0.61-0.80	2
2.21-2.40	5					0.81-1.00	2
2.41-2.60	0					1.01-1.20	3
2.61-2.80	1					1.21-1.40	1
Average 1.73 per cent.						Average 0.85 per cent.	
RASPBERRIES.							
Range. Per Cent.	No. of samples.						
1.21-1.40	10						
1.41-1.60	11						
1.61-1.80	12						
1.81-2.00	10						
2.01-2.20	5						
2.21-2.40	5						
2.41-2.60	0						
2.61-2.80	1						
Average 1.73 per cent.							



TABLE V.  
SHOWING NUMBER AND WEIGHT OF SEEDS PER 100 GRMS.

	No. of seeds per 100 grm.	Wt. of seeds per 100 grm.		No. of seeds per 100 grm.	Wt. of seeds per 100 grm.
GOOSEBERRIES	990	1.83	BLACKCURRANTS	3685	3.09
	540	1.29		5210	7.24
	730	0.97		4045	4.35
	410	1.03		4875	3.94
	320	0.97			
Average	600	1.22	Average	4450	4.66
RASPBERRIES	3565	4.11	BLACKBERRIES	1930	4.34
	3760	4.40		2700	5.57
	4900	5.85		3765	7.31
	4760	5.62		3450	8.01
	4050	4.37		3780	7.65
	4095	4.09		3460	7.63
Average	4190	4.74	Average	3180	6.75
REDCURRANTS	865	3.56	BILBERRIES	13,200	2.31
	940	4.29			
	1255	5.84			
	738	3.10			
	1055	3.86			
Average	970	4.13			

## Ministry of Agriculture and Fisheries.

### STATUTORY RULES AND ORDERS, 1929, No. 1117.\*

#### AGRICULTURAL PRODUCE (GRADING AND MARKING), ENGLAND.

#### THE AGRICULTURAL PRODUCE (GRADING) (POTATOES) REGULATIONS, 1929, DATED NOVEMBER 30, 1929, MADE BY THE MINISTER OF AGRICULTURE AND FISHERIES AS TO GRADE DESIGNATIONS FOR POTATOES.

In exercise of the powers conferred on him by the Agricultural Produce (Grading and Marking) Act, 1928, the Minister of Agriculture and Fisheries hereby makes the following regulations:—

1. Grade designations to indicate the quality of potatoes produced in England and Wales shall be as follows:—

E. & W. No. 1 Size  
E. & W. No. 2 Size  
E. & W. No. 3 Size

and the quality indicated by such grade designations shall be deemed to be as described in columns 2, 3, 4, 5, 6, 7 and 8 of the Schedule hereto.

2. These Regulations may be cited as the Agricultural Produce (Grading) (Potatoes) Regulations, 1929.

In Witness whereof the Official Seal of the Minister of Agriculture and Fisheries is hereunto affixed this thirtieth day of November, 1929.

(L.S.)

CHARLES J. H. THOMAS.

## SCHEDULE.

Definitions of Quality.		Applicable to Quantities.				
Grade Designation.	General.	Applicable to Single Tubers.		Tolerances.		
		Size (Minimum diameter).	Conformity to Variety, etc.	Undersize or Oversize.	Disease,* damage, etc.	Earth and/or extraneous matter.  <i>Maximum</i> aggregate of all defects under cols. 5, 6 and 7.
1. E. & W. No. 1 Size	2. Reasonably clean, healthy potatoes, free from serious defect and suitable for human consumption	3. 1½ in.†	4. At least 95 per cent., by count must conform to the variety as specified and to the type of soil on which grown, where such is declared.	5. Not more than 3 per cent. of the total weight may pass through a riddle or sieve having a square mesh of the minimum size† specified (in col. 3) for the grade, and, included in this, not more than 0.5 per cent. of the total weight may pass through a 1-in. mesh: potatoes which exceed 3¼ ins. in their smallest diameter shall be excluded. Otherwise, in regard to size, the potatoes shall be as grown.	6. Not more than 3 per cent. of the total weight may consist of appreciably diseased, damaged or unsightly potatoes, and, included in this amount, not more than 0.25 per cent. of the total weight may be obviously affected with soft rot.	7. Not more than 4 per cent. may be present in potatoes loaded up to November 1st in the year of harvesting, and 2 per cent. after that date the percentage to be calculated on the net weight of screened potatoes.
E. & W. No. 2 Size		1½ in.				8.
E. & W. No. 3 Size		1½ in.				

\* (i) Any disease or defect, the presence of which may be established by cutting open the potato, shall be taken into account, and potatoes having worm or slug holes penetrating into the flesh shall be regarded as damaged.  
(ii) Potatoes affected by superficial disease or damage shall not be regarded as diseased or damaged unless more than one-tenth of the surface is so affected.

(iii) A potato shall only be regarded as being obviously affected with the soft rot if, at the time of inspection, it is squashed and/or the surface is at some part distinctly broken or wet owing to disease.

† When the potatoes have been passed over a riddle of greater mesh than 1½ in., the minimum size may, at the seller's discretion, be appended to the grade name, e.g. E. & W. No. 1 Size (2 in.).

‡ As measured clear within the bounds of the mesh.

# Merchandise Marks Act

## MALT PRODUCTS.

### STATUTORY RULES AND ORDERS, 1930, No. 566.\*

#### THE MERCHANDISE MARKS (IMPORTED GOODS) No. 5 ORDER, 1930.

At the Court of Buckingham Palace, the 26th day of June, 1930.

PRESENT,

The King's Most Excellent Majesty in Council.

Whereas by sub-section (1) of Section 2 of the Merchandise Marks Act, 1926 (16 & 17 Geo. 5. c. 53), it is provided that after an enquiry in relation to goods of any class or description has on a reference from the appropriate department been held by a committee appointed for the purposes of the said Act, and the report of the committee on the matter has been taken into consideration by the department, that department may, unless it appears to them that the trade of the United Kingdom or the trade generally of other parts of His Majesty's Dominions with the United Kingdom would be prejudiced if imported goods of that class or description for use or consumption in the United Kingdom were prohibited to be sold unless they bear an indication of origin, make a representation to His Majesty that it is desirable that an Order should be made under the said Section 2, and His Majesty in Council may thereupon, subject to the provisions of the said Act, make an Order prohibiting the sale or exposure for sale in the United Kingdom of imported goods of that class or description unless they bear an indication of origin:

And whereas in accordance with the provisions of the said section an enquiry in relation to imported Malt Products has, on a reference from the appropriate department, namely, the Minister of Agriculture and Fisheries, the Secretary of State for the Home Department and the Secretary of State for Scotland acting jointly (hereinafter called "the Department") been held by a committee appointed for the purposes of the said Act and the report of that committee has been taken into consideration by the Department:

And whereas it does not appear to the Department that the trade of the United Kingdom or the trade generally of other parts of His Majesty's Dominions with the United Kingdom would be prejudiced if Malt Products imported for use or consumption in the United Kingdom were prohibited to be sold unless they bear an indication of origin, and the Department has accordingly made representations to His Majesty that it is desirable that an Order should be made under the said Section 2:

Now, therefore, His Majesty, by and with the advice of His Privy Council, in pursuance of the powers vested in Him by the said Act, and of all other powers enabling Him in that behalf, is pleased to order, and it is hereby ordered, as follows:—

1. For the purpose of this Order, the expression "malt products" shall mean malt extract, malt flour, malt extract and cod liver oil, and malt extract blended with any other product so that malt extract comprises more than 50 per cent. by volume of the whole.

2. It shall not be lawful to sell or expose for sale in the United Kingdom any imported malt products unless they bear an indication of origin.

3. The indication of origin shall be printed, stencilled, stamped or branded on the container, or on a label securely attached thereto, indelibly and in a conspicuous manner, in plain block letters not less than one-twelfth of an inch in height when the greatest dimension of the container does not exceed six inches, not less than one-eighth of an inch in height when the greatest dimension of the container exceeds six inches but does not exceed fifteen inches, and not less than one-half of an inch in height when the greatest dimension of the container exceeds fifteen inches. For the purpose of this Order, the expression "greatest dimension" shall mean the height, length or breadth, whichever is the greatest, of a rectangular or approximately rectangular container, and the height or maximum diameter, whichever is the greater, of a cylindrical, oval or conical container.

4. This Order shall apply on exposure for sale wholesale whether the person exposing the goods is or is not a wholesale dealer.

5. The provisions of this Order shall come into force at the expiration of three months from the date hereof.

6.—(a) This Order may be cited as "The Merchandise Marks (Imported Goods) No. 5 Order, 1930."

(b) The Interpretation Act, 1889, (a) shall apply to the interpretation of this Order as if it were an Act of Parliament.

M. P. A. HANKEY.

(a) 52-3 V. c. 63.

## Ministry of Health.

### REPORTS ON PUBLIC HEALTH AND MEDICAL SUBJECTS, No. 60.

#### THE EFFECT ON FOODS OF FUMIGATION WITH HYDROGEN CYANIDE.\*

HYDROGEN cyanide is being increasingly employed as a fumigant for the destruction of insects and vermin pests in food. Its advantages are absence of taint, freedom from injurious effects on textiles, paint-work and metals, high toxicity to insects, and ease of removal subsequently by ventilation. On the other hand, the smell is not sufficiently strong to serve as a warning that it is present in dangerous amounts, especially as individuals vary in their power of detecting its presence by sense of smell.

**METHODS EMPLOYED.**—These were discussed in an earlier report (No. 19, 1923) by Stock and the author. The older "pot" method, in which the gas was generated by the action of acids on cyanides (*loc. cit.*), is now superseded in most countries by liquid hydrogen cyanide. The name "Cyclon," formerly applied to a mixture of 90 per cent. of methyl cyanoformate and 10 per cent. of methyl chloroformate, is now transferred to liquefied hydrogen cyanide adsorbed on kieselguhr or infusorial earth, and supplied in sealed tins in admixture with sufficient lachrymator (ethyl brom-acetate) to act as a warning.

The liquid gas may be transported in steel cylinders if stabilised with a little acid, and it is also supplied in strong glass bottles with crown corks, and in metal drums with screw caps.

"Cyanogas" is a dry, grey-black powder consisting essentially of calcium cyanide, cyanamide and a little calcium carbide, which evolves hydrogen cyanide in moist air. Betaine tablets, which evolve the gas when heated, and a mixture with aluminium chloride of an addition-product of ferric chloride and hydrogen cyanide (which is liberated by water) are also used.

**RESIDUAL GAS IN FUMIGATED FOOD.**—This depends on (1) strength of gas, (2) duration of fumigation, (3) moisture content of food, (4) state of subdivision of food, (5) method of packing, and (6) subsequent ventilation.

In most of the published work the data are incomplete, since the concentration of gas used is usually recorded in terms of the weight introduced per unit space, without correction for the capacity of the contents, failure to produce gas-tight

\* By Dr. G. W. Monier-Williams. Obtainable at His Majesty's Stationery Office, Adastral House, Kingsway, London, W.C.2. Price 6d. net.

conditions, condensation, absorption and, in the case of the "pot" method, side-reactions. The only reliable data are obtained from analyses at the beginning of and during the fumigation process. Tables of results of other workers show the "nominal" concentration of gas, periods of exposure and ventilation, and parts of residual HCN per million. The last figures vary from 12 to 100 (water in open dish), 22 to 100 (milk), 8 to 1632 (cheese), 2 to 618 (oils and fats), 5 to 530 (meat and fish), 1 to 206 (cereals, flour), 3 to 580 (fresh fruit), 11 to 480 (dried fruit), traces in fresh vegetables (except 500 to 1100 in peas and beans), nil to 970 (tea, coffee, etc.). The author found nil to 3 in samples of grain from ships, and 63 to 102 and 24 to 47 for the top and centre layers respectively, of boxes of sultanas exposed for a few hours.

The variations are due to the widely different conditions used, but, in general, the results indicate the effectiveness of ventilation in removing excess gas (except in the cases of dried and liquid milk, dried fruit and, possibly, cheese). Absorption is greatest for moist, finely-divided unwrapped foods. Orange and apple skins resist absorption, whilst peaches and bananas are more readily penetrated. Cooking removes almost all the absorbed gas. In general, foods treated with less than 1 volume of hydrogen cyanide in 200 volumes of air, and subsequently exposed to air, retain less than 20 parts per million.

**TOXICITY.**—The fatal dose (Lehmann, *Chem. Ztg.*, 1915, 39, 573) is 60 mgrms. of hydrogen cyanide, though foods containing cyanogenetic glucosides have been known to produce death in quantities corresponding with 10 to 12 mgrms. It is difficult to suggest a limit, but there is no reason why a maximum of 20 parts per million should be exceeded for foods which are eaten raw, except in special cases (e.g. dried fruit), or when excessive local absorption may occur.

**CHEMICAL COMPOUNDS FORMED. ANALYSIS.**—Hydrogen cyanide may be retained in foods by laevulose, with which it forms a cyanhydrin. Dextrose cyanhydrin, however, forms only when the food is alkaline, whilst potassium cyanide disappears rapidly from foods containing either dextrose or laevulose. This is attributed to the formation first of the cyanhydrin, and then, by hydrolysis, in the presence of alkali, of hydroxy acids and ammonia, the latter reaction being favoured by the presence of alcohol (*cf.* Fischer, *Annalen*, 1892, 270, 64). There is little evidence, however, that glucose is a practical antidote to prussic acid poisoning. The toxicity of the cyanhydrins probably depends on the extent to which they may dissociate into sugar and hydrogen cyanide; but their formation is of importance, since it may serve as a means of storage in the food of hydrogen cyanide produced during fumigation.

These conclusions also indicate that, probably, the most satisfactory method of analysis is to promote dissociation by addition of water, and to distil in steam in a neutral or slightly acid medium. The author used 25 grms. of sample in 250 c.c. of water, and titrated the distillate with 0.02 *N* silver nitrate solution in the presence of sodium hydroxide and potassium iodide, the results being checked colorimetrically by means of the copper benzidine acetate reagent. The first 100 c.c. of distillate usually contained the whole of the hydrogen cyanide.

**Influence of Hydrogen Cyanide on Sulphur Dioxide Determinations.**—Attention is directed to the fact that the determination of sulphur dioxide in dried fruits, etc., is quite unreliable if the food has been fumigated for the destruction of insects and contains residual hydrogen cyanide.

**DETERIORATION OF FOOD ON FUMIGATION.**—Over-treatment may interfere with the natural respiration-processes of fruits and vegetables essential to their value as foods and so cause serious damage, and instances are given of softening



and discoloration. Since, under certain conditions, the gas has a stimulating effect on growth, there is evidence that a short exposure to a high concentration is preferable to prolonged exposure at low concentrations. The injurious effect is ascribed to the inhibitory action on the oxidation-reduction enzymes concerned with plant-respiration rather than to any direct action on the tissues themselves. Fermentative and proteolytic enzymes are less affected.

Fertile hens' eggs are killed by fumigation with 1 per cent. hydrogen cyanide (by volume) at 3° C. for 4 hours, although the amount actually absorbed is small.

ACTION ON BACTERIA, MOULDS, ETC.—These are relatively resistant to the gas, and are affected permanently only by excessive quantities, the use of which is precluded by other considerations.

J. G.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Determination of Aldose Sugars by Titration with Standard Iodine and Alkali Solutions.** G. M. Kline and S. F. Acree. (*Ind. Eng. Chem., [Anal. Ed.]*, 1930, 2, 413–415.)—A quantity of the sugar solution which will react approximately with 20 c.c. of 0.1 *N* iodine solution is neutralised, aqueous phenolphthalein solution being used as indicator; 5 c.c. of 0.1 *N* iodine solution are added, followed by 7.5 c.c. of 0.1 *N* sodium hydroxide solution. These additions are repeated until 22 c.c. of iodine solution and 35 c.c. of sodium hydroxide solution have been introduced. A definite quantity of 0.1 *N* hydrochloric acid is then added, and the liberated iodine is titrated with thiosulphate solution. After the addition of phenolphthalein solution the acidity is titrated with 0.1 *N* sodium hydroxide solution. If the liberated iodine requires more than 3 c.c. of 0.1 *N* thiosulphate solution, too much iodine has been added resulting in over-oxidation of the sugar; if less than 1.5 c.c. is required, insufficient iodine has been added. In either case, the determination must be repeated, adding more or less iodine as indicated; 0.15 gm. of xylose is equivalent to 20 c.c. of 0.1 *N* iodine solution or 30 c.c. of 0.1 *N* sodium hydroxide solution.

W. P. S.

**Chemistry of the Products of *Cocos Nucifera*. Part I.** J. P. C. Chandrasena. (*Biochem. J.*, 1930, 24, 1493–1495.)—In this paper, the first of a proposed series on the chemistry of the various products of the coconut palm, the author deals with the examination of the kernel and the oil at various stages of development of the nut, and also the constituents of the haustorium; no investigation has hitherto been carried out on the nature of the oil obtained. The following results were obtained with bunches of ripe nuts ready for harvest (*i.e.* nuts picked about a year after the complete setting of the fruit) from each of four trees, one from Colombo (Kitulwatta), the second from a suburb of Colombo

(Dehiwala), and the third and fourth from Moratuwa and Panadura, two coast towns 13 and 16 miles respectively south of Colombo.

Kernel.				Oil.	
		Water. Per Cent.	Oil. Per Cent.	Residue. Per Cent.	Sp. gr. at 29° C.
Kitulwatta	..	48.1	40.0	18.7	0.9152
Dehiwala	..	45.5	36.0	20.0	0.9297
Moratuwa	..	42.1	38.5	20.7	0.9190
Panadura	..	42.0	42.7	20.8	0.9170
Oil.				Residue.	
		Acid value.	Sap. value.	Iodine value.	Ash Per Cent.
Kitulwatta	..	0.42	234.1	6.84	5.87
Dehiwala	..	0.73	268.4	3.37	5.79
Moratuwa	..	0.23	260.3	6.48	4.86
Panadura	..	0.12	249.8	5.78	6.19
					Pentosans Per Cent.
					2.89
					1.96
					1.91
					2.10

The haustorium (coconut apple), which is yellowish on the surface but white inside with fibres running longitudinally, contained 84.9 per cent. of moisture, and the dried material yielded 18.9 per cent. of an oil which had sp. gr. 0.9177 at 29° C., saponification value 238.7, iodine value 16.1, and acid value 1.46. The press-juice of the fresh haustorium was an opaque liquid of sp. gr. 1.040, from which was extracted 0.62 per cent. of an oil with sp. gr. 0.9171, saponification value 248.0, iodine value 10.1, and acid value 1.47. The dry residual material, after extraction of the oil, contained: pentosans 9.49 per cent., lignin 27.0 per cent., and ash 4.05 per cent. The percentage of the water in the kernel was found to diminish with age, the iodine and acid values were high in the oils from the youngest bunches, and the iodine value was even higher in the oil from the haustorium. P. H. P.

**Variation in Colour-Test Value of Commercial Samples of Cod-liver Oil.** F. J. Dyer and F. Wokes. (*Quart. J. Pharm.*, 1930, 3, 417-426.)—Colour-test value is defined as the intensity of the blue colour produced by a saturated solution of antimony trichloride in chloroform on the oil under given conditions. Ninety grms. of antimony trichloride are rapidly weighed and transferred to anhydrous chloroform to make about 270 c.c., and the solutions used then have a sp. gr. of 1.626 to 1.627. The oil to be tested is dissolved in anhydrous chloroform to give a solution producing an initial blue colour between 4 and 8 Lovibond units (1.5 to 4 c.c. for the cod-liver oils used). To 0.2 c.c. of the freshly-made oil solution in a test tube of 1 cm. internal diameter are added at a given time 2 c.c. of the antimony solution, mixing is carried out by twirling, and the reading made in the tintometer exactly 30 seconds after mixing. If fading has commenced, readings are taken at 60 and 90 seconds, and by the curve of rate of fading the blue colour at maximum intensity is calculated. The values obtained on 24 commercial samples of cod-liver oil as sold to the public, and from various sources, ranged from 3.0 to 9.0, with an average of 5.7. From a study of these figures and others for oils as imported, the consumer in this country is apparently receiving an article

containing, on the average, only two-thirds of the activity (as measured by the colour test) of the satisfactory samples imported. The readings for oils stored under different conditions show that cod-liver oil is best preserved in completely filled amber-coloured bottles. A satisfactory sample of cod-liver oil should not give a colour-test value below 4.0. The practice of attempting to convert colour-test values into so-called biological units is regarded as unjustifiable. D. G. H.

**Fatty Acids in the Liver of the Sheep.** K. Turner. (*Biochem. J.*, 1930, **24**, 1327-1336.)—The fatty acids that can be isolated from the liver and other organs of animals differ from those that are found as glycerides in the adipose tissue, where reserve fat is held in store, but the precise nature of the unsaturated acids in these organs has been defined only in the case of certain acids from the liver of the pig. Hartley (*J. Physiol.*, 1909, **38**, 353) isolated an oleic acid from pig's liver in which he proved that the double bond was between  $C_{12}$  and  $C_{13}$ , and a linolic acid with double bonds between  $C_9$  and  $C_{10}$  and between  $C_{12}$  and  $C_{13}$ . No evidence of the presence in pig's liver of the ordinary  $\Delta^{9,10}$ -oleic acid was obtained, though this appeared to be the only oleic acid present in the adipose tissue fat of the pig. In a re-investigation of the nature of the oleic acid in pigs' livers containing about 4.5 per cent. of fat certain data have been obtained which point to the presence of both oleic acids in the organs used. An investigation has been made on the fatty acids obtained from the liver of the sheep. The ethyl esters prepared from these fatty acids were oxidised by the method of Armstrong and Hilditch (*J. Soc. Chem. Ind.*, 1925, **44**, 43T.), and a mixture of pelargonic and caproic acids was obtained. Oxidation of the mixed fatty acids with hydrogen peroxide according to the method described by Hilditch (*J. Chem. Soc.*, 1926, 1828) gave rise to dihydroxystearic acid, but not to tetrahydroxystearic acid. Dilute alkaline permanganate, however, produced a mixture of both these acids. The dihydroxystearic acid and tetrahydroxystearic or sativic acid were then oxidised separately. From the data obtained the oleic acid present in sheep-liver fat has been identified as the  $\Delta^{9,10}$ -modification which is commonly found in adipose tissue fat. Linolic acid (the same as Hartley obtained from pig's liver) is present, and also arachidonic acid, but there is no evidence of a linolenic acid. The occurrence of the linolic acid in the liver, but not in adipose tissue fat of the sheep, lends itself to the same interpretation as that given by Hartley for  $\Delta^{12,13}$ -oleic acid and linolic acid in pig's liver, *viz.* that it is formed from the  $\Delta^{9,10}$ -oleic acid by the introduction of a new double bond between  $C_{12}$  and  $C_{13}$ . P. H. P.

**National Mark Malt.** (*Pharm. J.*, 1930, **125**, 224.)—The contents of 32 jars selected at random (except that no recognisable brands were duplicated), from some hundreds of 1 lb. jars of malt extract with cod-liver oil, bought as "the kind generally sold," were analysed in the Government Laboratory for (1) protein content, (2) sp. gr., (3) diastatic activity of the malt extract, (4) volume of cod-liver oil, (5) and freedom from adulteration. (1) Two-fifths of the samples were below National Mark standard (5 per cent.) (see p. 45), two containing less than 4, nine between 4 and 5, and fifteen 5 per cent. and over, with a maximum of 6.9 per cent.

(2) Fifteen samples were below standard (1.4). (3) Only five samples conformed to the standard (25); nine samples were between 10 and 20, nine between 5 and 10; six below 5, and in two there was no diastatic activity. Only one sample complied with all the National Scheme requirements, not counting country of origin of the grain. (4) More than 50 per cent. were below National Mark standard; nine contained less than 13 per cent., and one only 10.8 per cent. of cod-liver oil.

D. G. H.

**Behaviour of Natural and Artificial Fruit Essences towards Sodium Paratoluene-sulphonchloramide (Heyden Chloramine). A. Miermeister.** (*Z. Unters. Lebensm.*, 1930, 59, 585-594.)—One c.c. of essence, 20 c.c. of water, 50 c.c. of a 0.01 *N* solution of chloramine-T reagent (Tillmans and Hollatz, *id.*, 1929, 57, 489), and 0.5 c.c. of acetic acid are kept for 4 hours in diffused daylight, potassium iodide and dilute sulphuric acid added, and the iodine titrated with 0.01 *N* sodium thiosulphate solution and starch indicator (*A*). Another 1 c.c. portion is shaken with 100 c.c. of water and 80 c.c. of ether, the filtered ethereal extract evaporated at the ordinary temperature, and the residue dissolved in 2 c.c. of alcohol and 20 c.c. of water, and the chloramine value (*B*) found as described above, the blank given by the reagent being deducted. The values *A* were determined for 22 natural and artificial essences, and with anise, vanilla, strawberry, raspberry, and mandarin, the ratios of the values for the natural to those for the artificial products were 12.3:0.9, 24.7:38.9, 6.8:29.0, 1.8:17.0, and 10.0:35.2, respectively. With volatile aromatic liquids the chloramine values of five 12 c.c. fractions, distilled from 1 c.c. of essence and 100 c.c. of water, were found. With essences of natural citrus fruits, apple, mandarin and orange, and artificial apple, banana, pear, apricot, anise and mandarin essences, the sum of the chloramine values of the fractions (which were always greatest for the first fractions) equalled the direct value *A*, but in the other cases *A* was the greater, the balance being accounted for by the chloramine value of the residue after distillation. The difference between the values *A* and *B* is, therefore, taken as the chloramine value of the non-volatile reducing substances. The results indicate that natural cocoa, coffee, anise, vanilla, currant, strawberry, and raspberry, and artificial strawberry, gooseberry, raspberry and vanilla essences contain only a small proportion of steam-volatile matter. Further directions are given for the treatment of various beverages containing these essences. The liquids should be filtered, and carbon dioxide removed by tossing. The reagent,  $\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2(\text{Na})\text{NCl}$ , is obtained from *p*-toluene sulphonic chloride, a by-product of the manufacture of saccharin, and is sold as a disinfectant under the names "aktivin," "mianin," "chlorazene," or "tolamine." It may also be prepared by sulphonation of toluene, treatment of the sodium salt of the sulphonic acid with phosphorus pentachloride, and conversion of the resulting sulphonic chloride into the sulphonamide by means of ammonia. The action of a slight excess of an alkaline 1.5 *N* solution of sodium hypochlorite then produces chloramine-T, which crystallises from the liquor. It forms colourless solutions stable for a month, but is decomposed by acid or alkaline hydrolysis into sodium chloride and oxygen.

J. G.

**Determination of Total Alkaloids in Cinchona Bark.** P. A. W. Self and C. E. Corfield. (*Quart. J. Pharm.*, 1930, 3, 410-416.)—The assay of cinchona bark, both by the B.P. and German Pharmacopoeia methods, is unsatisfactory, whilst that of the U.S.P. gives low and variable results. Extraction by ammoniacal alcohol is not recommended without preliminary treatment. The proposed method is to mix 10 grms. of bark (in about No. 60 powder) with a mixture of 7.5 c.c. of strong lead acetate solution and 12.5 c.c. of water, and leave for 1 hour. Fifty c.c. of ammoniacal alcohol (alcohol 97.5 and ammonia 2.5 parts) are then added, and, after standing for another hour, the mixture is extracted for 3 to 4 hours in a continuous extractor with boiling ammoniacal alcohol. The greater part of the alcohol is then distilled off, and 10 c.c. of *N* sulphuric acid and 40 c.c. of water added, and the mixture boiled and cooled. After filtration through cotton wool into a separator the mixture is treated with 10 to 20 c.c. of boiling 0.1 *N* sulphuric acid, cooled and filtered, and the flask and wool washed with cold acidified water until a few drops of filtrate show no opalescence with Mayer's reagent. Chloroform (20 c.c.) is added to the separator, the contents shaken for 2 minutes, and the chloroform layer run into another separator containing a mixture of 5 c.c. of *N* sulphuric acid and 15 c.c. of water. After shaking, the chloroform layer is rejected. The liquid in the first separator is shaken with two further quantities of chloroform, which are washed as before. The acid washings are transferred to the first separator, made alkaline with ammonia, and completely extracted with chloroform, the extract washed with water, the chloroform distilled off, 5 c.c. of alcohol added, and evaporation completed. The extract is then dried and weighed. It is of a high degree of purity, and results obtained by this method are accurate. D. G. H.

**Extract of Colocynth.** E. M. Smelt. (*Quart. J. Pharm.*, 1930, 3, 433-437.)—Extracts of colocynth were prepared with 60 per cent. alcohol and with diluted U.S.P. alcohol (48.4 to 49.5 per cent. by vol.  $C_2H_5OH$ ). The directions of the U.S.P. were followed, but the preparations could not be completed, since the amount of extract exceeded the final weight specified, *i.e.* 25 per cent. of the drug taken. Extracts were then made with 8 samples of colocynth pulp, and of these, 5 samples yielded over 25 per cent. of extract when 60 per cent. alcohol was used, and 6 samples over 25 per cent. with diluted U.S.P. alcohol. The samples giving lower values were an Egyptian colocynth several years old, and two brownish samples. It is suggested that the U.S.P. (10th Rev.) monograph requires revision. D. G. H.

**Assay of Stramonium Leaves and Tincture of Stramonium.** C. M. Caines. (*Quart. J. Pharm.*, 1930, 3, 342-348.)—The U.S.P. (10th Rev.) process for the assay of stramonium leaves is rapid and complete in the initial extraction, but the use of 0.2 *N* solutions throughout is regarded as preferable. The German Pharmacopoeia method is not recommended, nor are the methods of van Itallie, Eder, or the author regarded as satisfactory. A modification of the B.P. (1914) process (Caines, *Quart. J. Pharm.*, 1929, 2, 271) is suggested to overcome the

formation of obstinate emulsions, and this method is regarded as good. Ten grms. of the leaves, in No. 60 powder, are moistened with 5 c.c. of a mixture of 4 vols. of ether and 1 of chloroform, 2 c.c. of ammonia diluted with 3 c.c. water added, the whole packed into a small percolator, the resulting percolate distilled till the volume is 15 c.c., transferred to a separator, the flask washed with two quantities each of 5 c.c. of chloroform, 12.5 c.c. of 90 per cent. alcohol added, and the liquid extracted with successive quantities of 0.02 *N* sulphuric acid. The combined acid extracts are washed with 10 c.c. of chloroform (which is rejected), the liquid made distinctly alkaline with ammonia, and the alkaloids completely extracted with chloroform. The extracts are washed with 5 c.c. of water, the chloroform distilled off, and the residue dissolved successively in 3 c.c. of ether and 2 c.c. of absolute alcohol. The dried residue is dissolved in 1 c.c. of absolute alcohol, 5 c.c. of 0.2 *N* sulphuric acid added, and the excess acid titrated with 0.2 *N* sodium hydroxide solution, with methyl red as indicator, 1 c.c. of 0.2 *N* sulphuric acid being equivalent to 0.005785 gm. of alkaloid. The tincture (1 in 5) may be assayed by evaporating 100 c.c. to 10 c.c., transferring to a separator, and washing the dish with 2 c.c. of 45 per cent. alcohol, followed by 3 quantities of 5 c.c. of water, and then with 2 c.c. of ammonia, and finally with 4 quantities of 5 c.c. chloroform. After shaking, the chloroform layer is separated, the extraction repeated with two more portions of 10 c.c. of chloroform, 15 c.c. of 90 per cent. alcohol added, the chloroform solutions extracted with 0.02 *N* sulphuric acid, and the process completed as above. D. G. H.

**Determination of Nicotine in Oriental Tobaccos.** J. Burmann. (*Helv. Chim. Acta*, 1930, 13, 785-787.)—The following semi-micro-method demands extremely pure reagents, exact standard solutions, and accurately graduated burettes. The moisture in the tobacco is determined by heating 10 grms. of the tobacco, spread on a sheet of paper, in a ventilated steam oven at 100° C. for 3 hours, cooling and weighing. The dried tobacco is immediately ground to about the fineness of snuff and stored in a sealed vessel. To determine the nicotine, 3 grms. of the powder are triturated for some minutes in a porcelain dish about 10 cm. in diameter, with 3 c.c. of 30 per cent. sodium or potassium hydroxide solution by means of a glass rod. After 3 grms. of gypsum have been mixed in, the resulting dry powder is transferred to a 150 c.c. flask closed with a rubber stopper carrying a three-way tap. The flask is evacuated by a water pump, air being then re-admitted and the flask again evacuated, so that the bulk of the ammonia liberated is withdrawn. After addition of 75 grms. of absolute ether (freshly distilled over phosphorus pentoxide) the powder is shaken at intervals during an hour, then left for an hour or two and filtered rapidly through a pleated filter covered with a watch-glass. From 50 grms. of the clear liquid in a 150 c.c. Erlenmeyer flask the ether is distilled, by heating on a water-bath at about 50° C. The residue is treated with three successive quantities of 5 c.c. of the pure ether, each of which is distilled off. After all traces of ammonia have been expelled in this way, the residue is dissolved in 20 c.c. of pure ether, which is distilled off after addition of 50 c.c. of distilled water. The cold liquid is titrated with 0.01 *N*

hydrochloric acid in presence of 1 drop of a 1 per cent. solution of bromocresol purple in alcohol, using as comparison 50 c.c. of water containing one drop of the indicator and one drop of 0.01 *N* acid. Multiplication of the number of c.c. of the acid used by 0.081 gives the percentage of nicotine in the tobacco. The result may be checked gravimetrically by filtering the titrated liquid, washing on the filter, and making the volume of filtrate up to 100 c.c. This is mixed with 2 c.c. of concentrated hydrochloric acid (sp. gr. 1.19) and 3 c.c. of 12 per cent. silicotungstic acid solution. After the lapse of 24 hours the liquid is decanted off, and the precipitate collected, with the help of a pump, on a weighed Gooch crucible, and washed several times with water and then with alcohol and absolute ether. The weight of the precipitate, determined after drying at 100° C. and cooling in a desiccator over sulphuric acid, is multiplied by 0.0506 to obtain the percentage of nicotine in the tobacco.

T. H. P.

## Biochemical.

**Biochemistry of Aluminium. I. Excretion and Absorption of Aluminium in the Pig.** K. Mackenzie. (*Biochem. J.*, 1930, **24**, 1433–1441.)—The biological significance of aluminium is at present an unsolved problem; even when aluminium occurs in the diet in a readily available form, animals do not seem to utilise it in the metabolic processes to any extent. The utilisation of aluminium by young pigs has now been examined, and the path of excretion has been determined. An attempt has been made to ascertain if the excretion of urinary phosphorus is disturbed by the use of aluminised diets, and the amount of aluminium present in various organs has been determined. From the data obtained it is concluded that the intake and excretion of aluminium in the pig are equal (within the limits of experimental error). Aluminium excretion is confined solely to the alimentary tract. The absorption of aluminium from normal diets containing moderate amounts of available aluminium, as determined by the analysis of the organs, is small. Diversion of phosphates from the urine to the faeces does not occur on such diets; the urinary excretion of phosphate, therefore, disposes of the very doubtful assumption that aluminium may affect the metabolic processes by combining with available phosphate in the intestinal tract and thereby immobilising it. No harmful effect on general growth and metabolism results from feeding the pigs with comparatively large amounts of aluminium. The method does not take account of small quantities which might be absorbed and excreted without appreciably affecting the results.

P. H. P.

**Manganese in Relation to Nutrition.** M. B. Richards. (*Biochem. J.*, 1930, **24**, 1572–1590.)—The manganese content of a large number of substances of vegetable or animal origin has been determined. The analyses were carried out by a procedure based on the Willard and Greathouse periodate method, described by Richards (*ANALYST*, 1930, **55**, 554). The manganese content of plant reproductive organs has been investigated. Wide differences are found in different

plants, but there is little difference as regards manganese content between the male and female organs of the same plant. Analysis of lupin seeds at various stages of development shows that there is a marked increase in total manganese as the seed grows to maturity. This increase seems to bring further evidence that manganese may be regarded as an essential element for the development of the plant. The reproductive organs of water-loving plants, such as willow and sedge, show a high manganese content; it is of interest to note that a very high percentage of manganese has been reported in many true water-plants, such as *Zostera marina*. The data for the manganese content of foodstuffs indicate that, whilst soil conditions may have influence in determining manganese absorption by the plant, samples of a foodstuff from different sources show in general approximately the same content of manganese. From a consideration of the data for the manganese content of animal organs, the author concludes that, whilst we may be still in ignorance of the actual function of manganese in nutrition, there is a considerable amount of evidence in favour of regarding this element as one of the essential constituents of the animal organism. Direct proof of the indispensability of manganese for normal growth and development has not yet been furnished, and, in considering the possibility of its importance, it has to be remembered that manganese may behave in the animal body like silver, mercury, lead and other heavy metals, and that its presence in organs and tissues may have no real physiological significance. On the other hand, the steady increase of manganese in the developing egg, the invariable presence of the element in such tissues as the reproductive organs, and the constancy of the amount not only in these organs, but also in those most closely connected with the processes of assimilation (a constancy which is fairly well maintained when large amounts of manganese salts are added to the diet) are facts which suggest that manganese is no accidental constituent of the organism, but may have some intimate relationship to the vital processes.

P. H. P.

**Manganese in Foodstuffs.** A. E. Boycott and G. R. Cameron. (*Lancet*, 1930, 959.)—In searching for manganese as a possible cause of cirrhosis of the liver in drunkards, many foods and condiments were analysed for their contents of manganese. Manganese was absent from cockles, whelks and limpets; from marmite, tomato ketchup, essence of anchovy salad dressing, chutney, pickles (3 sorts), lobster paste, mustard, capers, and caraway seeds. Winkles contained, as mgrms. in 100 grms. of dried material, trace to 16; *Crepidula fornicata*, 10 to 17; oyster, 4; mussel, *nil* to 4; scallop, *Pecten opercularis*, trace to 40, *Pecten maximus*, 5; shrimp (bottled), *nil* to 4; prawn (bottled), *nil*; tea, 27 to 43; coffee, trace or *nil*; cocoa, trace; mixed spice, 14; cinnamon, 2; ginger, 4 to 12; coriander, 10; cloves, 48 to 50; vinegar, 1; curry powder, 4; olives (flesh), 6; pepper, 10 to 20; cayenne pepper, 2 or less. It is concluded that a vegetarian teetotaler would probably take in more manganese than a heavy drinker.

D. G. H.

**Destruction of Diastatic Enzymes in Honey on Heating.** H. W. Boer. (*Chem. Weekblad*, 1930, 48, 646-648.)—Honey may be heated for 24 hours at 60° C.



without destroying the diastatic enzymes, but at 65° C. the diastase value\* is reduced by 3 to 4 points, an average of 1 unit in 7 hours. Usually 12 hours' heating at 65° C. of a honey containing all the enzymes will not affect them much, but a similar heating of a honey which has been previously heated might destroy them all. The smaller the diastase value, the greater the risk that it will disappear on heating. Heating at 70° C. reduces the diastase value, on an average, 1 unit in 4 to 5 hours; at 75° C., 1 unit in 2 to 2½ hours; at 80° C., 1 unit in 10 minutes; at 90° C., 1 unit in 2 minutes; and at 95° C., 1 unit in ¾ minute, and the fall is most rapid at the beginning. (Cf. Lampitt, Hughes and Rooke, *ANALYST*, 1930, 55, 666.)  
D. G. H.

\* Gothe defines the diastase value of honey as the amount of 1 per cent. starch solution hydrolysed in 1 hour by 1 gm. of honey under the optimum conditions. The diastase values of the Dutch honeys examined ranged from 29.4 to 53.

**Vitamins of Olive Oil and the Effect of Refining.** G. Bertrand. (*Compt. rend.*, 1930, 191, 725-727.)—Virgin and refined olive oils were introduced into the diets of rats showing stationary weight curves for several days. An immediate rise in the curves was produced, but the weight increased much more rapidly with virgin than with refined oil. The difference is clearly manifest with the addition of as little as 3 per cent. of the oils, showing that the refining process impoverishes the olive oil of its vitamins.  
D. G. H.

**Question of the Identity of a Bacterial Growth-Promoting Factor with Vitamin B<sub>1</sub>.** J. G. Davis and J. Golding. (*Biochem. J.*, 1930, 24, 1503-1506.)—An investigation has been made of the vitamin B<sub>1</sub> content and bacterial growth-promoting properties of three commercial peptones. The test organism used was a lacto-bacillus requiring for good growth, not only the intermediate products of protein degradation, but also some factor present in all tissues containing vitamin B<sub>1</sub>. The vitamin B<sub>1</sub> contents of the peptones were compared by feeding tests on rats. The results showed that commercial peptones contain only negligible amounts of vitamin B<sub>1</sub>. There is no relation between the vitamin B<sub>1</sub> content of peptones and their ability to stimulate the growth of an organism requiring a vitamin B-like factor. Vitamin B<sub>1</sub> concentrates of the same order of potency for rat protection had widely different effects on the growth of the test organism. Since some had no effect at all, it may be concluded that vitamin B<sub>1</sub> itself has no influence on the metabolism of the organism. Therefore, the bacterial growth-stimulating substance and vitamin B<sub>1</sub> are not identical.  
P. H. P.

**Vitamin B<sub>2</sub> Content of Cereals and the Supposed Connection between Human Pellagra and Deficiency of this Vitamin.** W. R. Aykroyd. (*Biochem. J.*, 1930, 24, 1479-1488.)—Aykroyd and Roscoe (*Biochem. J.*, 1929, 23, 483) examined cereals and other foodstuffs for their vitamin B<sub>2</sub> content, with the use of the method of determination described by Chick and Roscoe (*Biochem. J.*, 1928, 22, 790). This work has now been further extended in order to continue the investigation of the relation between the epidemiology of human pellagra and vitamin B<sub>2</sub> deficiency. One of the most striking (and hitherto unexplained) facts

about human pellagra is its almost exclusive occurrence among maize-eating populations. In the previous experiments it was found that whole maize, though low in vitamin  $B_2$  when compared with foods containing animal protein, was by no means devoid of the vitamin, and that maize endosperm compared well with wheat flour in this respect. Two other staple cereals, rice and millet, have now been included, and their vitamin  $B_2$  content compared with that of maize. Two samples of whole rice and two samples of milled rice "raw" and "parboiled," were found to be poor sources of vitamin  $B_2$ . Whole millet is a poor source of vitamin  $B_2$ . Maize is a rather better source of vitamin  $B_2$  than millet or rice. Control rats from each litter were fed on the basal diet complete except for vitamin  $B_2$ . All remained practically stationary in weight, but skin symptoms did not consistently develop. Curative tests were made on two rats suffering from dermatitis. Diets which contained 65 per cent. of maize endosperm and 50 per cent. of whole rice respectively did not alleviate the symptoms. It is pointed out that, since neither rice nor millet shows any superiority over maize as a source of vitamin  $B_2$ , it is difficult to accept vitamin  $B_2$ -deficiency as the sole cause of human pellagra, as this disease is almost invariably associated with the consumption of maize. At present the association, if any, of vitamin  $B_2$  and human pellagra is obscure. The general distribution of vitamin  $B_2$  corresponds well with that of Goldberger's P-P (pellagra-preventive) factor, and the dietary factor which prevents "black-tongue" in dogs, as far as all three are known, and with the foodstuffs known from clinical observation to be preventive and curative of human pellagra, but the distribution of vitamin  $B_2$  as worked out on the rat, leaves the association of pellagra with maize unexplained, and gives no support to the theory that the dermatitis produced in rats by vitamin  $B_2$ -deficiency is the analogue of human pellagra. No satisfactory evidence has so far been obtained of the presence of a toxin in maize.

P. H. P.

## Bacteriological.

**Pyruvic Acid in Bacterial Metabolism with an Account of the Methods used for the Detection and Determination of Pyruvic Acid.** R. P. Cook. (*Biochem. J.*, 1930, **24**, 1526-1537).—For the qualitative detection of pyruvic acid the most sensitive and specific test is that of Posternak (*C. R. Soc. Phys. Hist. Nat. Genève*, 1927, **44**, (1), 519), in which the solution is treated with an equal volume of concentrated hydrochloric acid and a few crystals of phloroglucinol; a positive reaction (red colour) is obtained with 1 in 5000 pyruvic acid. For the quantitative determination the method of Wieland (*Liebig's Ann.*, 1924, **436**, 233) is the only one of use for small quantities. A new method is described for the determination which gives better results; it depends on the bisulphite-binding capacity of pyruvic acid. It may be used to determine acetaldehyde and pyruvic acid in a single sample. Friedemann, Cotonio and Shaffer (*J. Biol. Chem.*, 1927, **73**, 355; *ANALYST*, 1927, **52**, 418) applied it to the determination of acetaldehyde from the oxidation of lactic acid in the quantitative determination of the latter; the method for pyruvic acid is essentially the same. The method consists in

titration of the excess sodium hydrogen sulphite with iodine, and liberation of the bound sulphite with sodium carbonate. The liberated sulphite is then titrated with standard iodine solution, 1 c.c. of *N*/10 iodine being equivalent to 4.4 mgrms. of pyruvic acid. If acetaldehyde or other bisulphite-binding substance is present, the total bound sulphite is determined. Acetaldehyde or any substance of a volatile nature may be distilled off after hydrolysis with acid, and collected in bisulphite solution. The acetaldehyde may then be determined in the distillate. The pyruvic acid in the original solution may be determined by difference, and the result can be checked by very careful neutralisation of the original solution, addition of fresh bisulphite solution, and titration again of the bound sulphite. Pyruvic acid, even in small amounts, is quite stable in acid solution. For this method the use of ammonia-free water in the manipulations is absolutely necessary. Quantities as low as 0.2 mgrm. of pyruvic acid may be determined. Pyruvic acid is produced in the aerobic oxidation by *B. coli* of various substrates, if the reaction is checked by the use of fixatives for pyruvic acid. Pyruvic acid (as pyruvate) is not decarboxylated by *B. coli*. Evidence is brought to show that pyruvic acid is broken down by *B. coli* to a mixture of formic and acetic acids. P. H. P.

**Haemolytic Properties of Micro-Organisms belonging to the Paratyphoid Group.** C. P. Elliot and W. W. Ford. (*Amer. J. Hyg.*, 1930, **12**, 681-684.)—In an examination of 200 young rats infected with the virus of rat anaemia (*Bartonella muris*), 18 strains of paratyphoid bacilli have been isolated and identified by cultural and serological reactions, all the strains being Gärtner's *B. enteriditis*. They produce characteristic haemolytic colonies of the Alpha type of Smith and Brown on blood-agar plates made with rabbit erythrocytes. Haemolysins (haematoxins) are also found in broth cultures and in sterile neutralised Mandler filtrates of these cultures, and prove resistant to a temperature of 90° C. Haemolytic colonies have been observed also with other members of the paratyphoid group, including *B. paratyphosus B*, certain strains of *B. enteriditis* Gärtner, *B. aertrycke* De Nobele, *B. typhi murum* of Loeffler, and certain strains of Salmon and Smith's *B. cholerasius*. Some strains of *B. paratyphosus A* gave little or no haemolysis, whilst others gave the Alpha type. Haemolytic colonies were produced by two strains of *B. typhosus* and by strains of *B. coli* of human origin.

T. H. P.

## Toxicological.

**Methyl Alcohol.** (*Pharm. J.*, 1930, **125**, 234.)—At a conference on methyl alcohol in June at the U.S. Prohibition Bureau, Dr. Doran stated that the only jurisdiction the U.S. Treasury Dept. has over methyl alcohol (methanol) is as an ingredient of denatured alcohol, and alcohol containing as much as 10 per cent. of methanol has been consumed by the public. In the case of death from alcoholic poisoning the trouble appears to be due to ethyl alcohol until the mixture reaches 30 or 35 per cent. of methyl alcohol. Synthetic methyl alcohol now coming on

the market as a solvent, and as a 75 per cent. ingredient of certain anti-freeze solutions (of which some 40 to 45 million gallons per annum are used in the U.S.) seems likely to increase the danger.

D. G. H.

**Poisoning by Seeds of *Datura stramonium* (Thorn-Apple).** A. Sartori. (*Chem. Ztg.*, 1930, **54**, 890.)—The stomach, stomach contents, duodenum, and alimentary canal (total weight 135 grms.), in a case of suspected poisoning, were found to contain 54 brownish kidney-shaped seeds, 2–3 mm. in size, with wrinkled surface, these being identified as thorn-apple seeds. The following procedure served to isolate and identify the atropine: 150 grms. of a mixture of the organs supplied (including spleen, kidneys, liver and blood) were slightly acidified with tartaric acid and digested with alcohol for 24 hours at a moderate temperature. The alcoholic extract, showing green fluorescence, was evaporated on a water-bath and the residue purified by solution in water and alcohol alternately, with filtration of the various solutions. The final aqueous solution, which was almost clear, was shaken with ether and then, after over-saturation with sodium carbonate, with chloroform. The pale yellow residue resulting from evaporation of the united chloroform extracts at a low temperature was then tested as follows: (1) A drop of the neutralised solution of part of the residue in water containing a trace of sulphuric acid was introduced into the eye of a cat, the pupil showing dilation shortly afterwards. (2) Part of the residue was evaporated to dryness with a few drops of fuming nitric acid in a porcelain dish. Addition of a few drops of alcoholic potassium hydroxide solution to the cold residue yielded a violet coloration, soon changing to cherry-red. (3) A solution of the remainder of the residue in concentrated sulphuric acid exhibited frothing and emitted an odour of flowers when heated and treated with water.

T. H. P.

## Organic Analysis.

**Combustion of Methane by Means of Copper Oxide.** J. R. Campbell and T. Gray. (*J. Soc. Chem. Ind.*, 1930, **49**, 432–437T.)—Methane, prepared by the hydrolysis of magnesium methyl iodide, was passed through a 20 cm. column of closely-packed, fused, granulated sieved copper oxide (77.8 grms.). Since this occupied 18.9 c.c., the time of contact of gas and oxide could be calculated from the rate of flow of the former, the reduced oxide being regenerated in air after each experiment and used again. Circulation of the methane gave discordant results, and it was, therefore, passed over the oxide, maintained at the desired temperature, at a constant speed, the time of flow being noted, and the volume of gas determined from the volume of saturated magnesium chloride solution expelled from an aspirator. Since the products of reaction are carbon dioxide and water only, the percentage of the total methane oxidised is given by  $100 K / (K + M)$ , where  $M$  is the volume of methane in the gas after combustion and  $K$  the ratio of the amount of carbon dioxide ( $V$ ) to  $M$ . The results obtained by this and a method in which the gas was heated in intimate contact with the oxide in a closed tube and the reaction followed from the changes in pressure, showed that below 280° C.  $V$  is

almost negligible for moderate periods of contact. From 280° to 400° C. the rate of oxidation is slow, but at higher temperatures the amounts of methane oxidised tend to a maximum for any one temperature, and are influenced relatively little by the period of contact, so long as this is not small. Above 700° C. combustion is rapid, being complete in 7 minutes. Hence, when analysing gases by the fractional combustion method, it is necessary to oxidise hydrogen and carbon monoxide at temperatures not exceeding 300° C., if methane is the only other combustible gas present (*cf.* following abstracts, and Burrell and Oberfell, *J. Ind. Eng. Chem.*, 1916, 8, 228). J. G.

**Influence of Various Catalysts in Promoting the Oxidation of Methane by Means of Copper Oxide.** J. R. Campbell and T. Gray. (*J. Soc. Chem. Ind.*, 1930, 49, 447-450T.)—A mixture of nitrogen and 5 per cent. of methane was passed over copper oxide, the resulting carbon dioxide determined by absorption in alkali, and the amount of methane burned thence calculated. The oxidising agents used were: (1) copper oxide (8 to 32 mesh) activated by alternate reduction and oxidation. (2) copper oxide mixed with an aqueous suspension of cuprous chloride, and dried so as to contain 13.8 per cent. of  $\text{Cu}_2\text{Cl}_2$ ; (3) copper oxide and vanadium pentoxide, prepared by mixing the copper oxide with a suspension of ammonium vanadate in water, drying the mixture and igniting the residue; (4) copper oxide with 2 per cent. of cobaltous oxide; (5) copper oxide with 2 per cent. of nickelous oxide; (6) copper oxide with 2 per cent. of manganese dioxide. From the results the following conclusions were drawn:—(1) The rate of combustion is appreciable at 560° C. and complete at 705° C. (*cf.* preceding abstract) provided the contact-time is 2.4 to 3 minutes. The results at 808° C. indicate that an increase in rate of flow above a certain minimum produces a progressive decrease in the amount of methane burned, though very little methane is unoxidised even at the rate of 42.3 c.c. per minute. At 592° C. rates of 18.2 and 1.41 c.c. per minute produce oxidation of 21.6 and 94.1 per cent. of the methane, respectively. (2) Oxidation commences at 400 °C. and is complete at 475° C. Cuprous chloride loses its activity after prolonged use, probably as a result of fusion (m.pt. 430° C.), and hydrochloric acid is produced above 660° C. (3) Oxidation commences at 445° and 475° C., and is complete at 675° and 690° C. for 4.2 and 1 per cent. of  $\text{V}_2\text{O}_5$ , respectively, and the activity is not impaired by use. For (4), (5) and (6) the respective temperatures are 290°, 395° and 215° C. for commencement of combustion, and 650°, 675° and 675° C. for complete combustion, the contact times being 3 to 3.5 minutes. The mechanism of the action of cuprous chloride therefore differs from that of the other catalysts, and this catalyst may be considered ideal for the Dumas method of nitrogen determination (*cf.* following abstract). J. G.

**Oxidation of Various Gases by Means of Copper Oxide, Lead Chromate and Cobalt Oxide.** J. R. Campbell and T. Gray. (*J. Soc. Chem. Ind.*, 1930, 49, 450-453T.)—Addition of hydrogen or ethylene to methane lowers the external furnace temperature required for complete oxidation of methane in the presence

of copper oxide (*cf.* preceding abstracts), probably owing to the local generation of heat by the combustion of the more easily oxidised gas. The effect of ethylene, consequently, is greater than that of hydrogen, whilst its action as a negative catalyst or poison is not apparent in this reaction. Fused lead chromate is inferior to copper oxide in combustion analyses, since it may cause carbon monoxide to be formed as a result of incomplete combustion of the methane; moreover, lead chromate cannot be regenerated by oxidation *in situ*, even at 808° C. Cobalt oxide alone is equal in efficiency as an oxidising agent to copper oxide, whilst, in the presence of copper oxide, it has a positive catalytic effect (*cf. loc. cit.*). J. G.

**Separation of Alkyl and Aryl Halogen.** Q. Landis and H. J. Wichmann. (*Ind. Eng. Chem. [Anal. Ed.]*, 1930, 2, 394–397.)—Detailed methods are described for the determination of halogens in insecticides containing such substances as carbon tetrachloride, *p*-dichlorobenzene, etc. The Stepanow process for organic halogen is modified by heating a solution of the sample in kerosene or xylene with an excess of sodium; a small quantity of amyl alcohol is also added, and the heating is carried out under an efficient reflux apparatus. Benzenoid halogen is decomposed completely by this treatment. Aliphatic halogen in the presence of aryl halogen is determined by decomposition in kerosene solution with a concentrated potassium hydroxide solution in *n*-butyl or amyl alcohol. The mixture is heated at 100° C. in a pressure bottle for two hours, or at 115° C. for fifteen minutes. Total halogen in this case is determined by heating the mixture with sodium.

W. P. S.

**Pyrolysis of Vegetable Oils of Pronounced Acetyl Value (Grapeseed, Castor, Para Rubber Seed Oils).** R. Delaby and R. Charonnat. (*Compt. rend.*, 1930, 191, 1011–1012.)—The absence of ricinoleic acid from grapeseed oil is shown by the fact that, when decomposed by heating it under reduced pressure, such oil yields only traces of unsaturated aldehyde; saturated and unsaturated fatty acids of high average molecular weight (303–339) are obtained in 20 per cent. yield under these conditions. Pyrogenic decomposition of the sodium soap of castor oil gives sebacic acid, methylhexylcarbinol and the corresponding ketone, whilst similar treatment of grapeseed oil yields an insignificant alcoholic fraction forming an oily acid phthalate. Hence grapeseed oil contains no appreciable amount of ethylenic acid-alcohol capable of splitting into saturated aldehyde and unsaturated acid. Pyrolysis in a vacuum of oil from seeds of *Hevea brasiliensis* obtained from Indo-China yielded about 1 per cent. of liquid (b.pt. 80–140° C. at 14 mm. pressure) giving a faint coloration with Schiff's reagent, the bulk of the product being fatty acids, b.pt. 200–230° C., of mean molecular weight 312.

T. H. P.

**Para Rubber Seed Oil.** G. S. Jamieson and W. F. Baughman. (*Oil and Fat Ind.*, 1930, 7, 419–421, 437.)—The kernels of a sample of rubber seed (*Hevea brasiliensis*) yielded 42.53 per cent. of a dark red oil having the following characteristics:—Sp. gr., 25/25° C., 0.9185;  $n_D^{20}$ , 1.4737; saponification value,

191.8; iodine value (Hanus), 135.2; Reichert-Meissl value, 0.3; Polenske value, 0.2; acid value, 40.9; unsaponifiable matter, 0.8 per cent.; thiocyanogen number, 88.8; hexabromide value, 15.7; saturated acids, corrected, 16.0, unsaturated acids, corrected, 78.4 per cent. of iodine value, 163.8. The proportions of oleic (27.3), linolic (31.5) and linolenic acid (19.6 per cent.) in the oil were calculated by the Kaufmann method from the iodine value and the thiocyanogen iodine value of the oil. At least two isomers of linolenic acid are present. The saturated acids were separated by the lead salt and ether method and esterified, and six final fractions were analysed, showing the presence in the original oil of 7.3 per cent. of palmitic, 9.1 of stearic, and 0.3 of arachidic acid. The poor drying properties of commercial samples of rubber seed oil are regarded as due, at least partly, to the high acidity.

D. G. H.

**Para Rubber Seed Oil.** Y. Iwamoto. (*J. Soc. Chem. Ind. Japan*, 1930, 33, 409B.)—Fallen Para rubber seeds, collected from Malaya, were extracted with ether, and the oil tested for its acid value. That from fresh seeds was sweet to the taste, and had an acid value of 7.2, that from seeds not so fresh was somewhat bitter and of higher acid value, whilst that from putrified seeds was very bitter, and had an acid value of 110.86. Cold pressing gives a 28 per cent. yield of sweet edible oil, but hot pressing yields only 17 per cent. of a bitter oil from which the bitter taste may be removed by treatment with sodium hydroxide. The constants of the oil extracted from fresh seeds were found to be as follows:—Sp. gr. at 15°/4° C. 0.9234;  $n_D^{20}$  C., 1.4757; solidif. pt., 2° C.; acid value, 7.2; iodine value, 138.8; Reichert-Meissl value, 2.28; acetyl value, 2.41; unsaponifiable matter, 1.62 per cent.; liquid fatty acids, 80 per cent.; and solid fatty acids, 17.8 per cent. The liquid acids consisted largely of oleic, linolic and linolenic; the solid acids of 70 per cent. of stearic and 30 per cent. of palmitic acid. Various samples of crushed kernels were kept at room temperature for about three months and then extracted with ether. The acid value of the extracted oil was about six times as high as that extracted from the same kernels which had been previously heated for 5 hours at 105° C. The author concludes from this and other observations that the seeds contain a lipolytic enzyme.

R. F. I.

**Determination of Wax in Shellac.** A. G. Stillwell. (*Ind. Eng. Chem., [Anal. Ed.]*, 1930, 2, 387.)—Five grms. of the powdered shellac are dissolved in 150 c.c. of boiling water containing 3 grms. of sodium carbonate, the solution cooled, and the insoluble matter collected on a filter consisting of alternate layers of cotton-wool and asbestos, washed with 50 c.c. of 70 per cent. alcohol, and dried. The filter and its contents are then extracted with carbon tetrachloride in a Soxhlet apparatus, the extract is evaporated, and the residue of wax weighed.

W. P. S.

## Inorganic Analysis.

**Analytical Applications of the Reaction of Ammonia on Resorcinol in the Presence of Cations.** L. Bey. (*Bull. Soc. Chim.*, 1930, 47-48, 1192-1193.)—*Cadmium*.—A 5 per cent. solution of resorcinol in ether is added carefully to the

solution which has previously been treated with just sufficient ammonia to re-dissolve the hydroxide. A blue ring is produced at the junction of the liquids in 25 minutes in the presence of 0.001 per cent. of  $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ , but after 5 minutes it turns to the non-characteristic violet colour, also produced with smaller quantities of cadmium. Large amounts of copper inhibit the reaction, and, in the presence of small amounts, sufficient potassium cyanide should be added to remove any blue colour due to cadmi- and cupri-ammonic ions. *Tin*.—The solution is oxidised by means of bromine water, shaken with 2 c.c. of 5 per cent. ammonia, and 2 c.c. of a 5 per cent. aqueous solution of resorcinol added. The liquid above the precipitated stannic hydroxide appears blue in 2 to 20 minutes. With less than 0.0015 per cent. of stannous chloride a non-characteristic green-grey colour results. Antimony chloride does not affect the result, unless the proportion of antimony is 400 times that of the tin; in that case the antimony should be separated with hydrogen sulphide in the usual way (*cf.* ANALYST, 1929, 54, 561). J. G.

**Titration of Lead Salts.** R. C. Wiley, P. M. Ambrose and A. D. Bowers. (*Ind. Eng. Chem. [Anal. Ed.]*, 1930, 2, 415–416.)—The lead solution to be titrated should not contain other metals which form insoluble molybdates. It should be neutralised with ammonia, and any precipitated lead hydroxide dissolved by boiling the solution after the addition of ammonium nitrate. The solution is boiled until it is neutral and, while still hot, titrated with standardised molybdate solution. The end-point is indicated when a drop of the mixture yields a brown coloration with a drop of pyrogallol and chloroform solution. W. P. S.

**Detection of Tellurium in Bismuth.** H. Töpelmann. (*Z. anal. Chem.*, 1930, 82, 284–295.)—The spark spectrum of tellurium gives few sensitive lines; the two strongest (2385.8 and 2383.3Å) are in the ultra-violet. The most delicate reaction is the stannous chloride test, which detects 0.002 mgrm. of tellurium in 1 grm. of bismuth; an almost immediate brown discoloration is produced. Arsenic interferes, as it gives the same reaction (Bettendorff's test); selenium gives a red precipitate. Hence the stannous chloride test is not sufficiently specific. The author combines the above two reactions, thereby obtaining a specific and more sensitive test. The metal is dissolved in nitric acid, the solution evaporated to dryness on the water-bath, and the residue heated with sulphuric acid till white fumes are evolved. The mass is taken up in hydrochloric acid of sp. gr. 1.13 (10 c.c. for 1 grm., 50 c.c. for 10 grms. of bismuth). 0.001 grm. of arsenic as arsenite (collector for tellurium), and 5 c.c. of 10 per cent. stannous chloride solution in hydrochloric acid (sp. gr. 1.13) are added; the dark precipitate is left to deposit overnight, collected, washed with 1 per cent. stannous chloride solution followed by water, and dissolved in strong nitric acid. The solution is concentrated by evaporation, rinsed into a 4 c.c. beaker, and taken to dryness. The residue is dissolved in a drop of hydrochloric acid and this transferred to a hollow carbon electrode, the beaker being washed twice with a drop of acid. The spark is then passed and the appearance of line 2383.3 in the spectrograph observed (exposure



15 minutes). Proceeding in this manner the author was able to detect 0.00005 per cent. of tellurium in 10 grms. of bismuth.

W. R. S.

**Electrometric Titration of Chromium in Steel and Ferro-chrome. F. Spindeck.** (*Chem. Ztg.*, 1930, **54**, 890.)—With steels containing high percentages of chromium, determination of the latter by reduction with ferrous sulphate and titration with permanganate does not give trustworthy results, and iodimetric titration is more costly. The following electrometric method gives satisfactory results. The apparatus required consists of a Fischer's electrical stirrer, two platinum electrodes, an Emich filter rod or a leg cut from an electric plug, which is easier to fill than the filter rod, and a micro-ammeter with resistance. The filter rod is filled with a solution prepared by dissolving 2.926 grms. of ammonium vanadate and 4.9 grms. of ferrous ammonium sulphate in water acidified with 50 c.c. of sulphuric acid (1:5) and making up to 500 c.c. From 0.3 to 0.5 gm. of the metal is dissolved in 1:5 sulphuric acid, the solution being then oxidised with silver nitrate and ammonium persulphate solution, and the permanganic acid reduced with 1:1 hydrochloric acid. After cooling, the liquid is treated with manganese sulphate solution and titrated with ferrous ammonium sulphate solution. The whole of the resistance is inserted at first, so that the galvanometer needle reaches about the zero point of the scale. The resistance is then cut out to give the greatest sensitivity and the titration carried out dropwise to the end-point, where the needle shows a large deflection. The end-point may be confirmed by again inserting the resistance; the needle then travels back over the scale and scarcely moves when 1–2 drops of the ferrous ammonium sulphate are added.

Material difficult to dissolve is fused with sodium peroxide, dissolved in water, and the solution made up to a definite volume. An aliquot part is filtered off, acidified with sulphuric acid, and at once titrated with ferrous ammonium sulphate. Tungsten, nickel, and cobalt do not interfere with the titration.

T. H. P.

**Identification of Aluminium and Magnesium in Printing Inks. L. M. Larsen.** (*Ind. Eng. Chem. [Anal. Ed.]*, 1930, **2**, 416–417.)—The ink is mixed with a quantity of linseed oil varnish and a small amount of cobalt drier, and the mixture spread evenly on paper which has not been treated with alum during its manufacture. When the ink is quite dry, strips of the paper are boiled for one minute with 2 *N* hydrochloric acid, and the solution is decanted and cooled. If not more than a trace of iron is present, the solution is treated with an equal volume of "aluminon" reagent (250 grms. of ammonium acetate, 10 grms. of glacial acetic acid and 1 gm. of aurin tricarboxylic acid in 1000 c.c. of water), the mixture boiled for one minute, and cooled. A deep red flocculent precipitate indicates the presence of aluminium. The precipitate remains unchanged when the mixture is shaken with an equal volume of ammoniacal ammonium carbonate solution. To test for magnesium, strips of the paper are boiled for two minutes with 5 per cent. acetic acid, the extract is decolorised, if necessary, by heating it with the addition of a few drops of 2 per cent. chloramine-T solution, cooled, and treated with an

equal volume of 5 per cent. sodium hydroxide solution. One drop of an aqueous 0.025 per cent. benzopurpurin 4B solution is then added. A rose-red precipitate indicates the presence of magnesium. W. P. S.

**Determination of Calcium and Magnesium in Dolomitic Limestones by means of Saccharate Solutions.** A. C. Shead and B. J. Heinrich. (*Ind. Eng. Chem. [Anal. Ed.]*, 1930, 2, 388–389.)—The method depends on the solubility of calcium hydroxide in sucrose solution and on the insolubility of magnesium hydroxide or oxide in this solvent. About 0.5 grm. of the sample is ignited at 900° to 1000° C. until the weight is constant, the oxides are then transferred to a flask and heated to boiling with 25 c.c. of water; the mixture is cooled while the flask is closed to prevent the entrance of carbon dioxide, 100 c.c. of 30 per cent. sucrose solution are added, and the mixture is shaken thoroughly. The insoluble magnesium hydroxide (oxide) is collected on a paper-pulp filter under conditions which exclude atmospheric carbon dioxide, washed several times with dilute sucrose solution, then transferred to a flask, boiled with the addition of an excess of 0.2 N acid, and the excess of acid is titrated, phenolphthalein being used as indicator. The calcium is determined indirectly by boiling a weighed quantity of the sample with an excess of 0.2 N acid, titrating the excess of the acid, and deducting the alkalinity due to the magnesium hydroxide as found in the first titration. W. P. S.

**Determination of Beryllium in Aluminium.** H. V. Churchill, R. W. Bridges and M. F. Lee. (*Ind. Eng. Chem. [Anal. Ed.]*, 1930, 2, 405–407.)—One grm. of the alloy is dissolved in 25 c.c. of 1:1 hydrochloric acid, the solution is treated with hydrogen sulphide, filtered, the filtrate is evaporated until crystals appear, an equal volume of ether is added and dry hydrogen chloride is introduced until the two phases are completely miscible. The precipitate is collected on a filter, washed with a mixture of concentrated hydrochloric acid and ether saturated with hydrogen chloride, dissolved in a small quantity of water, and reprecipitated with hydrogen chloride as before. This precipitation may be repeated once more if necessary. The combined filtrates are evaporated, the residue is heated with 5 c.c. of 1:1 sulphuric acid until white fumes are given off, cooled, dissolved in water, filtered, and the filtrate neutralised with ammonia, rosolic acid being used as indicator. The mixture is boiled, filtered, the precipitate washed with ammoniacal ammonium chloride solution, dissolved in hydrochloric acid and reprecipitated, again dissolved, the solution nearly neutralised with ammonia, heated at 60° C., and treated with an excess of 8-hydroxyquinoline and ammonium acetate solutions. The mixture is filtered, the filtrate treated at 60° C. with a slight excess of ammonia, cooled, the precipitate collected, washed with ammonium acetate solution, dried, ignited, and weighed as beryllium oxide. W. P. S.

**Determination of Nitrous and Nitric Acids in Sulphuric Acid.** H. A. J. Pieters and M. J. Mannens. (*Z. anal. Chem.*, 1930, 82, 218–224.)—The subject was re-investigated. Lunge's nitrometer, which gives excellent results with large

quantities of nitrogen, is no longer reliable when the nitrogen concentration is less than 0.0005 grm. per c.c. *Nitrous acid*.—The best results are obtained by the colorimetric *m*-phenylenediamine method; permanganate titration is reliable if no other reducing substance is present, but this does not seem to be the case with crude chamber acid. *Nitric acid*.—The authors reject colorimetric determination with brucine and favour the following reduction method. The acid (50 c.c. for 0.0001 to 0.0005 grm. nitrogen per c.c.) is introduced into a small separating funnel, the tube of which dips into 200 c.c. of water in a conical flask well cooled in running water; the acid is allowed to run slowly into the flask containing the water, followed by an excess of potassium hydroxide in strong solution. After addition of 3 grms. of Devarda's alloy, the flask is immediately connected with a receiver through a cooler, and the ammonia distilled into a measured excess of standard acid. This operation gives total nitric and nitrous nitrogen; the latter is given by the phenylenediamine process, and the former computed by difference.

W. R. S.

## Microchemical.

**Colorimetric Micro-reactions of the Glutogenic Protides and Cellulosic Gels of the Wheat Grain.** P. Bruère. (*Compt. rend.*, 1930, 191, 792–794.)—Treatment of a transverse section of a wheat grain with a 0.1 per cent. solution of bromocresol green (yellow for values of *pH* below 3.6 and blue for those above 5.2) in neutral 60 per cent. alcohol reveals compact blue masses of glutogenic protides in the cells fused to the innermost of the six membranes of the skin, termed the seat of the enzymes by Bertrand. In the middle of the corn, these masses become less compact. When a flour is used, the blue glutogenic masses, starch granules and cellulosic debris may be squeezed out by means of the cover-glass, blue, pasty, amoeboid lumps, corresponding with the peptised gel known as moist gluten being then observed. When use is made of bromocresol purple, which is yellow for *pH* values not exceeding 5.2 and purple-violet at the neutral point, the colorations obtained indicate, with the ripe corn, increase in acidity from *pH* 6.8 at the periphery to *pH* 5.6 at the centre, the mean being about 6.2. Flour recently milled shows the value 6, although this value varies with the degree of extraction, chlorination, ageing, etc.

T. H. P.

**Erratum.**—In the abstract of the paper by Wagenaar (*ANALYST*, 1930, 55, 349), for "cystine" read "cytisine."

## Physical Methods, Apparatus, etc.

**Emulsification. Part III. A Factor Inhibiting the Emulsification of Cod-liver Oil.** E. Lester Smith. (*Quart. J. Pharm.*, 1930, 3, 373–374.)—A substance which stabilises water-in-oil emulsions and inverts or reduces the stability of oil-in-water emulsions is produced in cod-liver oil during oxidation or

drying. The extent to which the oxidation has been carried in an oil may be found by the "drop weight method," which is a measure of the interfacial tension. A small pipette, with very fine capillary constriction above and below the bulb, but terminating in a fairly coarse jet, is filled to the upper constriction with water, and the jet lowered into a beaker of oil. The flow of water is started by blowing, and the number of drops formed while the pipette empties to the lower capillary is counted. This number (which is inversely proportional to the surface tension) divided by the volume of the pipette, gives the number of drops per mil. Figures recorded for a number of oils varied from 4 to 23. Most good oils give values of 9 or under.

D. G. H.

**Quantitative Spectral Analysis.** P. Urbain. (*Bull. Soc. Chim.*, 1930, 47-48, 1183-1188.)—The method depends on the electrolytic deposition of the trace of metal to be determined on a rod or wire of metal giving no spectral lines which may interfere with those due to the ultimate rays of the metal to be determined. If, in addition, a known quantity of a substance is added which produces lines close to the ultimate rays of the metal sought, the spectrograph then shows (1) the ultimate rays of the metal sought, (2) the principal rays of the comparison metal, and (3) rays due to the electrodes and impurities in the electrolyte, etc. Pairs of lines (doublets) may then be made up from a line of the metal sought and the nearest line of the comparison metal, and their intensities compared from the depths of the corresponding serrations in the curve obtained when the plate is examined micro-photometrically. The line of total opacity given by the points of commencement and conclusion of the photometric curve is taken as the reference-line. Since the quantity of comparison metal deposited simultaneously with the metal sought is constant, the ratio of the degrees of blackening of the plate for the doublets concerned is a measure of the amount of the latter metal. The error depends on the efficiency of electrolytic deposition, and micro-photometric measurement, and is usually less than 10 per cent. The technique for the determination of gold with silver as comparison metal (or *vice-versa*) is described. Solutions containing (say) 0.05 mgrm. of silver and 1 to  $50 \times 10^{-3}$  mgrm. of gold are deposited from 0.1 *N* potassium cyanide solutions on carbon rods 2 mm. in diameter, with platinum anodes (3 volts, and 1 milliamp. per sq. cm.), the containing vessels being rotated to ensure thorough mixing. After 4 hours, spectrographs are taken with a discharge of 10,000 volts across electrodes 2 mm. apart, and the spectrograph plates divided into several sections, each of which corresponds with a doublet, e.g.  $\lambda$  (2676, 2660), (2428, 2438), (3123, 3281), etc., for gold and silver, respectively. Typical photometric curves of such plates are shown and discussed.

J. G.

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## Reviews.

ALLEN'S COMMERCIAL ORGANIC ANALYSIS. Fifth Edition. Edited by C. AINSWORTH MITCHELL, M.A., D.Sc., F.I.C. Volume VIII. Pp. x+761. London: J. & A. Churchill. 1930. Price 30s.

The new "Allen," when complete, will apparently consist of nine volumes instead of eight, as in the previous edition. Most readers will probably agree that the arrangement of articles in this edition has been improved, but naturally in the last two volumes the more or less miscellaneous groups are bound to accumulate, and so we pass from the homogeneous volumes VI (Dyes and Colouring Matters) and VII (Alkaloids) to this rather heterogeneous volume, which deals with groups so diverse as bitter principles, enzymes, cyanogen compounds and proteins, and the editor has made the best arrangement possible of this variegated material.

The first three chapters are by Dr. Julius Grant on glucosides, bitter principles, and enzymes. Although a great deal of work has been done on all three subjects in the sixteen years that have elapsed since the previous edition was prepared, Dr. Grant has been able to fit the new material neatly into the old framework, except in the case of enzymes, where it has been necessary to expand the article from fifteen to forty-two pages, and to add much new matter.

The chapter on bitter principles has needed less revision, except as regards hops, which, in view of recent work, has been largely re-written. It would be interesting to know whether there is any real evidence of the presence of even traces of morphine in American wild hops (p. 117). It is almost fifty years since the statement was made, and nobody seems to have verified it.

The section on glucosides has been largely extended and, as in the two sections just alluded to, the revised and added matter is all of a kind to make the chapter more useful to the analyst, particularly such new matter as the biological standardisation of the heart-stimulating glucosides (p. 40), the summary of information on the estimation of prussic acid yielded by plant-products containing cyanogenetic glucosides (p. 14), and the resumé of recent work on saponins (p. 52). It is not easy to see what kind of a glucoside could be isolated by the following process (p. 10). "Sometimes it is quicker to steam-distil an aqueous extract of the bark and to precipitate the distillate with lead chloride and lead oxalate."

Though Dr. Grant is clearly aware of the useful English and American practice of calling the crystalline glucoside of *Strophanthus gratus*, ouabain, and so distinguishing it from amorphous strophanthin ex *S. Kombe*, he does not make this distinction clear in his description of strophanthin (p. 48), which is the place where it is most wanted.

Professor Barger suggests that putrefaction bases should no longer be called ptomaines, and provides, in thirteen pages, a brief but clear summary of information on these interesting substances.

The publishers have been generous, almost lavish in fact, in the space allocated to constitutional formulae in Dr. Falk's article, on "Animal Bases," and the reproductions of photomicrographs of crystals with which he has replaced some of the more diagrammatic illustrations of the previous edition are particularly good. This article, like that by Drs. Hawk and Bergeim on "Animal Acids," has clearly been revised with great care. Mr. Buchanan's article on "Cyanogen Compounds" not only admirably fulfils its primary purpose of catering for the needs of the analyst, but, incidentally, provides an interesting illustration of the immense part played by such compounds in modern civilisation, and the meticulous care with which chemists have devised means for the control of the various agricultural and industrial applications of these dangerous substances.

The last two chapters on "Proteins" and "Digestion Products of the Proteins" were started by the late Prof. Schryver and finished by his collaborator Dr. H. W. Buston. They consist of the informative and practical articles, which Dr. Schryver contributed to the previous edition, thoroughly revised and brought up-to-date, and they will undoubtedly be of great value to anyone who has to deal with analytical problems in this complex and difficult group of products.

This is a volume on which the Editor and his collaborators can be cordially congratulated.

T. A. HENRY.

COCOA AND CHOCOLATE MANUFACTURE. By H. W. BYWATERS, D.Sc., F.I.C.  
Pp. xii+316. London: J. & A. Churchill. Price 21s.

As the author remarks, books dealing with cocoa and chocolate are unusually scarce; they are also very incomplete. We have here, however, a work which publishes a large amount of useful information, especially as to methods and machinery. The book is the culmination of many years' observations from an important vantage point, and the laboratory evidently had a satisfactory link with manufacture. The independent setting out of cocoa powder and chocolate manufacture into two sections, complete in themselves, enables the non-expert reader to grasp the real sequence of the complex operations and their various alternatives.

To the works' chemist and analyst the most disappointing feature is the devotion of a mere 11 pages out of 307 to the laboratory tests and figures for cocoa products. These omissions make the book much less useful for reference in the chemists' library than it might have been; no doubt, many interests are involved. Considering first the laboratory applications: one hoped for a full set of figures recording the many physical and chemical values of varieties of cocoa butter, which subject has practical application. We have, however, to be content with a quotation of the values published by an allied laboratory. The general statement

as to the differences of Arriba and Accra cocoa butters would be of more interest if specifications of production were given.

Like most of us, the author seems to have overlooked the significance of the work of H. Fincke (1921) on the phosphoric anhydride variation (*i.e.* lecithin) in cocoa butter, although the fact is now made use of in manufacture. The repetition here of the high average fat content (53 to 55 per cent.) for cocoa mass, and roast nib, has not been confirmed in the writer's laboratory, where the best Soxhlet determinations have given the lower range 52–54 per cent. The rather high melting point recorded for cocoa butter (93°–96° F.) must be due to the method used—a cold-filled capillary tube.

The big subject of cocoa butter substitutes receives a very slight description. These fats are in large and permanent use, and their control requires the constant aid of the laboratory.

It is a reflection on our chemical knowledge of cocoa that only thirteen pages are here allotted to the constituents and their changes. Even so, we are glad of some authoritative details of tannin-body investigations. The reviewer, however, cannot accept the repetition of the very low tannin determinations of Adams (1.0–2 per cent.), without proof that extraction was complete.

The analyst will look in vain for those figures of ash composition of varied cocoas which are useful for identification and for determining adulteration, process, etc. Public analysts and others will note the author's decisive views as to cocoa alkalisation, which he denies is injurious in the slightest degree. Even so, it is scarcely necessary to claim that such alkaline action on protein "cannot be other than helpful to their digestion." Alkalisation is, in fact, an advantage to the consumer, and is carried out by the manufacturer at a substantial cost.

It is very notable that for shell determinations reliance is placed on crude fibre determination, the eccentricities of levigation not being accepted. It is not quite clear what is meant by the statement that only a fraction of shell present may be detected by any known "chemical" method after super-grinding.

Considering now the main theme of the book, manufacture—as might be expected, the sections on cocoa powder production—roasting, alkalisng, pressing and grinding—are of great value, and even the advanced practices of uniform roasting after husk stripping, and continuous roasting, are described. Valuable basic data are given throughout. The "liquoring" of cocoa powder, with cold mixing and milk addition, for testing as a beverage, is not the only method used by experts. Although solvent fat extraction is noted, the technical use of absolutely fat-free cocoa for certain food purposes is not mentioned.

In the manufacture of chocolate the somewhat opposed aims of fineness and optimum flavour production are also discussed in all their bearings. Some of us, who are aware of the older technique of many English manufacturers, will not be cheered at the thought of competing with the advanced machine products of the

Continent and the United States. The physical condition of chocolate is throughout well treated, although some numerical values for the viscosity changes would have been appreciated.

In addition to the usual views on the effects of conching, the author makes the further suggestions that loss of bitter (?) taste, occurs with improvement in colour, as a result of full conching. The reviewer, however, cannot confirm these changes. Some chemical analyses of the possible tannin changes, presumed both by the reviewer and the author, would be useful knowledge. The writer is able to confirm Dr. Bywaters' lack of preference for cane sugar in chocolate, and is of opinion that good beet sugar answers every requirement of the palate.

The extreme abbreviation of the technology of milk chocolate is unfortunate, as it is the most important home product. Neither the stages nor the variations are given with the fullness which is essential for so sensitive a manufacture. The technical data given as to enrobing are satisfactory, so far as they go, but they overlook other practices used in the attainment of fine and stable surface. As indicated, a great deal of information may be obtained as to radical improvements in manufacture—especially by those competent to develop them. Finally, one may envy the author the concise and natural way in which he sets out his knowledge for our benefit. To the expert the book is indispensable.

H. R. JENSEN.

HANDBUCH DER KAUTSCHUK-WISSENSCHAFT. By Prof. K. MEMMLER with Co-workers. Pp. 747 and Index. Leipzig: S. Hirzel. Price: Stitched, M.57.50; bound, M.60.

This work is, perhaps, the most comprehensive which has yet appeared on the various aspects of the technology of rubber. The subject-matter is treated mainly from the theoretical standpoint. It is the work of seven experts in their own branches of the subject, and is accordingly divided into seven sections dealing, respectively, with (A) Botanical and Cultural Aspects, (B) Chemistry, (C) Vulcanisation, (D) Chemical Analysis, (E) Physics, (F) Physical or Mechanical Methods of Testing, and (G) Microscopic Examination.

Speaking broadly, the book is well up-to-date, and the subject-matter of each section is fully treated, with very few omissions. Some of the sections are excellent. Sections "A" and "B," by Prof. Zimmermann and Prof. Pummerer and Dr. Koch, respectively, strike me as being particularly good. In section "E," by Professor Hock, we have, for the first time, a succinct account of the more recent physical work on rubber from the pen of one who has himself contributed several papers in recent years on this subject. Sections "D" and "F" are those of most interest to readers of our journal. Section "D," by Prof. Kindscher, is perhaps the least satisfactory in the book. It is rather of the nature of a catalogue of methods of analysis, and it does not read as if the author were in the habit of making frequent use of the operations he describes. In this respect it is much less interesting than the previous section "C" by the same author.



In contrast to section "D," section "F," by Profs. Memmler and Schob, dealing with the mechanical tests, does read as if the authors were perfectly familiar with the details of design and working of the machines and appliances described. They have definite opinions, boldly stated, which command attention, although one may not in all cases see eye to eye with them. Thus, they describe the figure for "tensile product" as a false measure, as it takes no account of the character of the load-stretch curve. It may be at once admitted that this criticism is perfectly valid. On the other hand, the "tensile product" as an approximate figure is of considerable value, and the relative figures for similar types of rubber approach very closely those of "proof resilience," which is admittedly a more accurate representation. The latter, however, necessitates the determination of an area bounded by a curve which must be plotted, and it is easy to see that, even with autographic records, the time taken in working out the figures for the large number of determinations which are necessary in technical work would hardly be possible. This section, particularly, is worthy of careful study. The last section, by Dr. Pohle, is one of the most interesting. In spite of obvious difficulties, this method of attack may become increasingly important.

There are, as might be expected, the usual defects of treatises of this type. Some overlapping occurs which, however, could hardly be avoided, and disproportion in the space allotted to different subjects, although, naturally, different portions of a book with such a wide range will interest different persons to a widely varying extent. There are occasionally misprints, particularly with the initials of authors, but, on the whole, the work is well arranged, clearly printed, and packed full of references. The only complaint on this score is the weight of the paper, and consequently the book is too heavy to support in the hands; unbound, it tends to fall to pieces.

H. P. STEVENS.

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

### The Determination of the Milk Proteins.

By GEORGE M. MOIR, M.Sc., Ph.D., A.I.C.,

*Pedler Research Scholar of the Institute of Chemistry, 1928-1930.*

*(Read at the Meeting, November 5, 1930.)*

#### II. THE IDENTITY OF THE CASEIN PRECIPITATE.

INTRODUCTION.—Recent extensive experiments which have been carried out by Linderstrom-Lang<sup>1</sup> have raised the question of whether casein is one substance or several. By heating casein with various solvents (60 per cent. alcohol at about 60° C. with or without  $N/1000$  HCl, and also dilute HCl-NaCl solution) he has been able to separate it into fractions differing in various respects, notably in their phosphorus content. The interpretation to be placed upon these experiments depends upon whether the effect of the solvent is truly and only physical, or whether it is accompanied by some slight chemical action between the protein and the solvent. It is quite to be expected that in a substance like casein, with a molecular weight in the vicinity of 200,000,<sup>2</sup> or even more,<sup>3</sup> there may be quite large different groups that are held together in the molecule by comparatively weak linkages, so that they are capable of being separated by mild treatment. Only if the solvent applied to casein acts in a purely physical manner can it be correct to say that casein is not homo-molecular. Linderstrom-Lang admits that "It would seem as if no especial clearness has been brought by the results obtained, which instead of simplifying the chemistry of casein apparently complicate it"; but he also summarises his results thus: "Casein is a hetero-molecular substance, a more or less homogeneous mixture of several sorts of

colloidal molecules, components which *on account of mutual interaction* follow along through the processes to which casein is, in general, subjected, and thereby *form a co-precipitation system.*" (I have italicised two phrases.) The "mutual interaction" may quite possibly be of the nature of residual valencies or bonds, which, it has been suggested, play an important part in linking together portions of the large molecules of proteins. The large number of  $-\text{CO.NH}-$  groups which are supposed to be present in proteins could very easily be the sites of such residual bonds, which might be readily broken by comparatively mild chemical treatment. Subsequent experiments may confirm the idea that casein is hetero-molecular, but the experiments so far carried out seem to me to come short of proving it. In the absence of proof, the important thing to remember, for the present purpose, is that casein certainly does form a very definite co-precipitation system.

The foregoing discussion draws attention to the desirability of producing evidence that the material precipitated from milk by the proposed analytical method is really casein and nothing but casein. The problem thus raised of the composition and characterisation of a genuine protein substance is not a simple one, a fact which was emphasised not many years ago by one of the foremost modern protein chemists, S. P. L. Sørensen.<sup>4</sup>

FUNDAMENTAL CONSIDERATIONS.—One physical property of proteins that is of especial value here is their isoelectric point. In a review of the "Physical Chemistry of the Proteins," Cohn<sup>b</sup> wrote: "The concept of the isoelectric point has a two-fold origin. Born of colloidal chemistry, it has acquired a theoretical significance and a mathematical definition in terms of electrolytic dissociation"; and "The minimum solubility should coincide with the isoelectric point on the simplest assumptions that can be made."

Casein is a protein which is typically insoluble at its isoelectric point, and it is to be separated in milk from two other proteins which are typically soluble, even at their isoelectric points, in salt solutions such as exist in milk. Cohn later (p. 402) says of casein, "The precipitation of this protein at this point is remarkable, and its purification depended upon its relative insolubility under these conditions long before the theory of the isoelectric point had been developed." The analytical proposal, first made by Waterman, to precipitate the casein from milk at a  $p\text{H}$  as close as possible to its isoelectric point, has thus an obvious fundamental basis, and one might even go so far as to define casein as the material which is precipitated from milk at this particular  $p\text{H}$  (4.6) by means of acetic acid and sodium acetate. The necessity for specifying the buffer to be used is obvious from the experiments of Michaelis.<sup>5</sup>

Before discussing methods of investigating the casein precipitate, some consideration may, with advantage, be devoted to the precise nature of the problem. The improved analytical method which I have evolved gives figures which are usually about 1-2 per cent. higher than those obtained by the use of acetic acid alone. The additional material which is thus precipitated must be either casein or one of the other milk proteins. If it is casein, then the protein precipit

pH 4.6, and at pH 4.2 must have the same chemical properties. If the higher values obtained at pH 4.6 are due to contamination with, say, lactalbumin, then methods, which are incapable of showing differences due to as little as 1-2 per cent. of the contamination, cannot be used. For this reason several possible methods must be ruled out.

#### EXPERIMENTAL.

Investigation of the phosphorus-nitrogen ratio of the precipitate at first sight appears attractive, but it is complicated by the fact that very great difficulty has always been found in removing from a casein precipitate the last traces of inorganic calcium and phosphorus which are combined with it in the milk. When casein is precipitated at different H-ion concentrations it is not to be expected that the inorganic impurities will be removed to the same extent, and so a factor will be introduced which will interfere with the value of phosphorus determinations. When some analyses were carried out, this expectation was confirmed.

The method of dissolving the casein and reprecipitating it was suggested as a possible means of discovering whether the precipitate was contaminated with soluble protein material. When this was tried by dissolving the casein in dilute sodium hydroxide solution, lower amounts of re-precipitated protein were certainly obtained, but unfortunately this evidence cannot be relied upon. The reason for these lower values is that the action of dilute alkali has been shown by Carpenter<sup>6</sup> to split off soluble material from the casein. On account of this and other important changes<sup>7</sup> which are produced in this protein by alkali, it can be quite definitely asserted that after casein has been subjected to even faintly alkaline pH values it is no longer the same material as it was before.

A number of difficulties stand in the way of using the specific rotation of alkaline solutions of the casein. Racemisation is liable to occur and alter the values. Some experiments were carried out to see if the method used by Woodman<sup>7</sup> and Wright<sup>8</sup> to identify various proteins by means of their racemisation curves might be used to examine the casein precipitated at different pH values. The method was abandoned because of the fact that exceedingly opalescent solutions of casein were obtained, so that the accurate readings required could not be made. The opalescence was, no doubt, due to the presence in the solution of traces of calcium phosphate. To obtain reliable readings it was found essential to have the temperature of the solution in the polarimeter tube very carefully controlled.

**FORMOL TITRATION METHOD.**—Another method of obtaining a curve which shows the progressive decomposition of the protein by alkali solutions, in a manner similar to the racemisation curve, is that proposed by Abderhalden and Kroner.<sup>9</sup> This consists in measuring the increase of amino-nitrogen by means of the formol titration. For the purpose of the experiment casein precipitated from skim milk at two pH values was dissolved in sodium hydroxide solution and made up to a definite volume, so that the strength of alkali in each case was normal. After pipetting portions for Kjeldahl determinations of the amounts of casein dissolved,

the flasks were placed in a thermostat at 37° C., and portions of 10 ml. were removed at intervals for formol titration. For this purpose, the casein solution was neutralised in each case by adding carefully from a pipette the same definite quantity of normal hydrochloric acid, drop by drop, so that precipitation of the casein was avoided. The formol titration was carried out by a modification of the method of Northrop.<sup>10</sup> The solution of formalin (5 ml. containing thymol blue) was added to the neutralised casein solution and the mixture titrated colorimetrically to pH 8.8 with *N*/20 sodium hydroxide solution. The figure thus obtained was taken as the basis for the results which appear in the following table:

TABLE I.

Casein precipitated at pH 4.14 0.4403 grm. <i>N</i> per 100 ml. milk.		Casein precipitated at pH 4.61 0.4518 grm. <i>N</i> per 100 ml. milk.	
Time from start. Hours.	Amino- <i>N</i> in per cent. of total <i>N</i> .	Time from start. Hours.	Amino- <i>N</i> in per cent. of total <i>N</i> .
0	10.35	0	10.2
$\frac{1}{2}$	13.6	$\frac{1}{2}$	11.9
1	13.5	0 $\frac{1}{2}$	14.3
1 $\frac{1}{2}$	14.7	1 $\frac{1}{2}$	14.9
2	15.2	1 $\frac{3}{4}$	15.25
3	16.0	2 $\frac{1}{2}$	16.2
4 $\frac{1}{2}$	18.35	3 $\frac{1}{2}$	16.5
6	20.0	5	18.4
8	21.0	7	20.0
20 $\frac{1}{2}$	26.2	19	25.3
27 $\frac{3}{4}$	28.1	27	28.1

When these figures are plotted on a graph the points from both caseins lie close to a single curve. Since Abderhalden and Kroner have shown that different proteins give distinctly different curves, the two samples of casein (which were precipitated from the same skim milk) appear to be identical. The slight deviations of the points from the curve are due to errors of the method. A normal soda solution is neutralised by a normal acid solution before proceeding to titrate with *N*/20 sodium hydroxide solution. An error of 1/200 ml. in the pipetting of either of the two first solutions would give rise to an error of 0.1 ml. in the final titration, or about 0.3 per cent. of amino-nitrogen. Much better results were obtained by neutralising always with the same definite pipetted quantity of acid instead of trying to adjust colorimetrically to pH 7.0. The results would probably have been still better if the titrations had been carried out electro-metrically.

**ALKALINE HYPOBROMITE OXIDATION METHOD.**—Goldschmidt and Steigerwald<sup>11</sup> have described a method which enabled them to distinguish clearly between different proteins—gelatin, casein and albumin. The method depends on the fact that, owing to structural differences, the proteins react differently with alkali hypobromite and, as in the racemisation and formol titration methods, distinctly different curves are obtained. Of the three methods the hypobromite is much the easiest to use.

The hypobromite solution was prepared by dissolving 2.66 ml. of bromine in 250 ml. of (approx.)  $N/2$  sodium hydroxide solution. To avoid the possibility of this solution reacting with the milk fat, skim milk was used to provide the casein. The casein nitrogen content of the milk was determined by means of acetic acid alone at  $pH$  4.23, and found to be 0.405 grm. of  $N$  per 100 ml.; the improved method at  $pH$  4.65 gave 0.410 grm. of  $N$  per 100 ml. Portions of 100 ml. of the same milk were precipitated at these two  $pH$  values and, after being filtered and washed in the usual way, the casein obtained was used for the experiment. A part of each sample was used to prepare two solutions in such a way that each contained exactly the same amount of casein nitrogen, the solution of which was effected by the addition of 25 ml. of  $N/9$  sodium hydroxide solution. The volume was made up to 200 ml., which contained 0.152 grm. of  $N$ .

TABLE II.

0.152 grm. casein $N$ and 25 ml. $N/9$ NaOH per 200 ml.				0.247 grm. casein $N$ and 20 ml. $N/9$ NaOH per 200 ml.			
Casein pptd. at $pH$ 4.23.		Casein pptd. at $pH$ 4.65.		Casein pptd. at $pH$ 4.23.		Casein pptd. at $pH$ 4.65.	
Time from start	$N/10$ NaOBr used per grm. $N$ .	Time from start	$N/10$ NaOBr used per grm. $N$ .	Time from start	$N/10$ NaOBr used per grm. $N$ .	Time from start	$N/10$ NaOBr. used per grm. $N$ .
Minutes.	Ml.	Minutes.	Ml.	Minutes.	Ml.	Minutes.	Ml.
2½	53.6	3	62.8	2	57.8	2	61.3
9	90.3	9	97.1	9½	93.5	8½	93.2
18	121.4	20	133	19	121.4	22½	131.3
41½	160.1	53	185.6	37½	154.4	39	160
57½	183.8	78½	213.2	59	183.4	61	187.2
130	245.8	125	246	114	227	119	229
270½	298	272½	304	250	282	264	284
352	322	384	330	354	307	359	307.6

In preliminary trials the reaction was found to proceed very slowly at  $0^{\circ}C.$ , the temperature used by the original workers, so that for the experiment a thermostat at  $19^{\circ}C.$  was used.

Two 200 ml. dry flasks, each containing a dry 10 ml. pipette, had previously been placed in the thermostat. After using each pipette to determine accurately the strength of the sodium hypobromite solution by pipetting it into excess of acidified potassium iodide solution with  $N/10$  thiosulphate, the same pipette was used to transfer 40 ml. of the hypobromite solution into the flask. Ten ml. of water and 25 ml. of  $N/9$  sodium hydroxide solution were also added, so that the addition of 25 ml. of the casein solutions made a total volume of 100 ml. At intervals 10 ml. were pipetted from each flask into potassium iodide solution acidified with ample hydrochloric acid, and the unused hypobromite thus estimated. In Table II appear the results obtained from the two casein solutions, together with other figures obtained in exactly the same way from solutions of the same samples of casein, but of different strength—0.247 grm. of casein  $N$  dissolved

in 20 ml.  $N/9$  of sodium hydroxide solution and made up to 200 ml. When these figures are plotted on a graph two curves are obtained corresponding to the different concentrations of protein and alkali in the solutions used. This and other experiments which I have carried out show that it is not possible to make an accurate comparison between two samples of casein unless the concentrations of protein, alkali and hypobromite are the same in the two reaction mixtures being compared.

This condition is fulfilled in each of the two separate parts of this experiment, but not for all four reaction mixtures, hence the two curves. In the first part of the experiment the values from the two caseins (precipitated at  $pH$  4.23 and 4.65) fall on one curve, and in the second part, where the solutions differed in strength from those used in the first part, the values from both caseins fall along another curve. From this it may be concluded that the same protein material is precipitated at both H-ion concentrations.

SUMMARY.—1. Upon the fundamental basis of the conception of the isoelectric point, it has been suggested that casein be defined as the material which is precipitated from cows' milk at  $pH$  4.6, by acetic acid buffered by sodium acetate.

2. Two methods have been used to show that casein precipitated in this way is chemically identical with the material precipitated at  $pH$  4.2 by acetic acid alone.

(a) When the two caseins are digested with normal caustic soda at  $37^{\circ}C.$ , the production of amino-groups, as determined by the increase of the formol titration, takes place at the same rate.

(b) The rate of oxidation of the two caseins by sodium hypobromite under comparable conditions is the same for both.

This paper represents part of the work carried out during the author's tenure of the Pedler Research Scholarship of the Institute of Chemistry, the assistance of which and the interest of the members of the Pedler Fund Committee is gratefully acknowledged. I wish to add my appreciation of the facilities made available to me at the National Institute for Research in Dairying, and especially of the valuable advice of Capt. J. Golding, Head of the Chemical Department.

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UNIVERSITY OF READING.

(Part III, "Proposed Modified Method for Casein" will be published in the March issue.)

## The Hydroxylamine Method for the Determination of Ketones.

### Carvone in Caraway and Dill Oils.

BY C. T. BENNETT, B.Sc., F.I.C., AND T. TUSTING COCKING, F.I.C.

THE method described by Bennett and Salamon (ANALYST, 1927, 52, 693) has been applied by us to several aldehyde- and ketone-containing oils with a view to its adoption as a general method. Our experiments show that a uniform method is not applicable in all cases, and that the method requires modification according to the particular ketone present.

In the case of aldehyde-containing oils, the modified method recommended by the Essential Oil Sub-Committee for the determination of citral in oil of lemon (ANALYST, 1930, 55, 109) was found to be of almost universal application, provided that a little benzene was added as a solvent for the non-aldehydic portion of the oil.

This modified method was found to be quite unsuitable for the determination of ketones; the reaction took place very slowly, and did not proceed to completion, even after many hours' contact with the reagent.

The reagent in this case was  $N/2$  hydroxylamine hydrochloride solution in 60 per cent. alcohol; a small excess only was used, and the reaction was carried out in the cold, with continuous shaking.

In order to hasten the reaction, the use of a stronger alcohol (80 per cent.), as in the method used by Bennett and Salamon, was tried as a solvent; in alcohol of this strength the oil was soluble, and distinctly better results were obtained, but the reaction was still too slow, taking about 24 hours for completion. Methyl orange, the indicator recommended in the case of oil of lemon, where 60 per cent. alcohol is the solvent, is unsuitable in stronger alcohol, and dimethyl yellow (dimethyl-amino-azo-benzene) was used instead. This has the same colour change and works over the same pH range as methyl orange.

The experiments with the 80 per cent. alcoholic solutions were repeated, the determinations being made at a temperature of about 70° C., and a vast improvement resulted. The reaction was complete in about 60 minutes, and the end-point was fairly distinct, but it was found somewhat difficult to get concordant results with different operators.



The following results were obtained on several ketone-containing oils by varying the conditions:

#### THE DETERMINATION OF KETONES BY HYDROXYLAMINE.

##### DILL OIL.

By sodium sulphite method,

	57 per cent. by volume = 60.3 per cent. by weight of carvone				
„ cold hydroxylamine method, 30 minutes	= 45.5	„	„	„	„
60 minutes	= 52.8	„	„	„	„
18 hours	= 57.2	„	„	„	„
24 hours	= 58.7	„	„	„	„
„ hydroxylamine method,					
heated to 70° for 30 minutes	= 58.2	„	„	„	„

##### CARAWAY OIL.

By sodium sulphite method,

	57 per cent. by volume = 60.3 per cent. by weight of carvone				
„ cold hydroxylamine method, 30 minutes	= 44.6	„	„	„	„
60 minutes	= 51.8	„	„	„	„
18 hours	= 55.8	„	„	„	„
24 hours	= 56.8	„	„	„	„
„ hydroxylamine method,					
heated to 70° C. for 30 minutes	= 56.8	„	„	„	„

##### PENNYROYAL.

By sodium sulphite method, 84 per cent. by volume	= 83 per cent. by weight				
(4 hours)					
„ cold hydroxylamine method after 2 days	= 82.4	„	„	„	„
„ hydroxylamine method boiling for 1 hour	= 87.1	„	„	„	„
(under reflux)					
„ hydroxylamine method, heated to 70° C. for					
90 minutes	= 86.5	„	„	„	„

##### PIPERITONE.

1. By sodium sulphite method (1 hour in water-bath)	= 97 per cent. by volume				
2. By hydroxylamine method, 70° C.					
1 hour	= 75.4	„	„	„	„
1½ hours	= 91.9	„	„	„	„
2 hours	= 97.7	„	„	„	„

After numerous experiments it was found that more uniformity was obtained when a still stronger alcohol (90 per cent.) and a stronger reagent were used, and when a larger excess of the reagent was present. Eventually the conditions for the determination of carvone in the oils of caraway and dill were standardised, and concordant results, agreeing well with those obtained by neutral sulphite absorption, were obtained.

The method finally adopted is as follows: About 1.5 grms. of the oil are weighed into each of two stoppered tubes, and to each tube are added 10 c.c. of approximately *N* hydroxylamine hydrochloride reagent, and the tubes are placed in a beaker of water on a boiling water-bath, the temperature rising to about 75° C. The colour of the solutions changes from yellow to red owing to liberated hydrochloric acid, and at 5 minute intervals the tubes are removed from the water-bath,

and neutralised with *N* alcoholic potash contained in two separate burettes. As the reaction proceeds it becomes slower, and towards the end the indicator does not become red, but only a golden orange, and the exact end-point is a little difficult to judge without practice. When it is thought that the end has been reached, the readings of the two burettes should be taken, and then to one of the tubes an additional 0.5 c.c. of the alcoholic potash should be added. This titration is now definitely over-run and the full colour of the indicator is developed. If this colour is a paler yellow than that of the other tube, it is evident that this has not been titrated to the full yellow colour, and the titration is continued until it is exactly the same colour as that in the tube with the excess of alkali. The calculation of the result is based on this latter titration. The reaction is complete in about 35 to 40 minutes.

During the titration a certain amount of potassium chloride is precipitated, but this does not interfere with the result nor make the end-point more difficult to see.

The reagent is prepared by dissolving 6.95 grms. of pure hydroxylamine hydrochloride in 95 c.c. of 90 per cent. alcohol, adding 0.4 c.c. of a 0.2 per cent. solution of dimethyl yellow and sufficient *N* alcoholic potash to bring the reaction of the reagent to the *full* yellow colour of the indicator.

This adjustment is best carried out by dividing the solution into two portions and adding alcoholic potash to each in turn until the last drop produces no change in colour; about 0.8 c.c. is required. The addition of the alkali produces a turbidity due to precipitation of potassium chloride, but, on shaking, this slowly redissolves. As the reaction of the reagent, and of the solution at the end of the test, is more alkaline (or, rather, less acid) than pure hydroxylamine hydrochloride, it is necessary to introduce a small factor as a correction.

This is accomplished by multiplying the theoretical figure by 1.008. 1 c.c. of *N* potassium hydroxide solution is therefore equivalent to  $0.1501 \times 1.008$  grm. = 0.1513 grms. of carvone.

#### RESULTS OF DETERMINATIONS OF CARVONE IN CARAWAY OIL.

By neutral sulphite and acetic acid 54.5 per cent. (v/v) = 57.8 per cent. (w/w).

By *N*/2 hydroxylamine reagent in 90 per cent. alcohol.

2.5 c.c. excess reagent used, 2 hours, temperature about 75° C., 57.1 per cent.

"	"	"	"	58.4	"	"
"	"	"	"	58.4	"	"

By the method finally adopted, using 10 c.c. of *N* hydroxylamine reagent in 90 per cent. alcohol.

Equivalent to approximately 100 per cent. excess, 50 minutes 58.9 per cent.

"	"	"	"	40 minutes	58.5	"	"
"	"	"	"		58.7	"	"
"	"	"	"		57.6	"	"
"	"	"	"	29 minutes	58.2	"	"

Further determinations carried out by different observers 58.2 per cent.

58.4	„	„
58.4	„	„
58.5	„	„
58.7	„	„
58.9	„	„

The accuracy of the results was confirmed by determinations carried out on pure carvone. Using 10 c.c. of the reagent and about 0.8 grm. of the carvone, the results obtained were 99.3, 99.8, 99.9, and 100.3 per cent.

On a mixture of the above pure carvone and a pure terpene, the method gave results within  $\pm 0.2$  per cent. of the calculated figure.

The advantages of this method over the neutral sulphite method are several:—  
(1) Only a small quantity of the oil is required. (2) Duplicate determinations are carried out at the same time. (3) The result can be obtained in less than an hour.

The method has been applied to the determination of ketones in other oils; menthone in oil of peppermint, and pulegone in oil of pennyroyal. In both these cases the end-point was found to be difficult to see, owing to the darkening of the oil itself, when heated with the reagent, obscuring the colour-change of the indicator. Instead of a pure yellow colour being obtained at the end, the colour was golden orange, and the result was uncertain to the extent of several per cent. It is hoped that further research will result in improvements and modifications of this useful method whereby it may be applied to all ketone-containing oils.

The experimental work in connection with the above has been carried out in the laboratories of The British Drug Houses, Ltd., and Wright, Layman & Umney, Ltd., and we are indebted to our Directors for permission to publish our results.

## The Lead Reduction Method for the Volumetric Determination of Tin and the Interference with it by Copper and Antimony.\*

By S. G. CLARKE, B.Sc., Ph.D., A.I.C.

*(Read at the Meeting.)*

THIS paper contains the results of an investigation of the determination of tin by reduction from the stannic condition by means of lead and direct titration with iodine in presence of the lead, a process which was first introduced by A. R. Powell

\* Communication from the Research Department, Woolwich.

in 1918. It is shown that by keeping the solution during the reduction and titration in an atmosphere of carbon dioxide, for which purpose an apparatus already described by B. S. Evans was used, quantitative results can be obtained when the amount of tin is calculated from the absolute amount of iodine used. This is the case even with small amounts of tin down to 0.5 mgrm., which is the practical lower limit when 0.01 *N* iodine is used, no modification of the standard process used for the higher amounts being required beyond the use of the more dilute iodine solution as the titrating liquid. The investigation was extended to cover the effects on this method for tin, of copper and antimony, which have been generally supposed to have no appreciable influence. The effect of copper, however, is profound, the results for tin becoming markedly lower in proportion as the copper is increased, and this is due to the precipitation of tin in the form, it is believed, of a compound with copper. The effect of antimony is less marked, but distinct.

**REDUCING AND OXIDISING AGENTS.**—Innumerable methods have been published based on the reduction of stannic tin by metals followed by an oxidising titration; almost all of the common metals have been advocated as reducing agents by one author or another, and the same is true of iodine, ferric chloride, etc., as oxidising agents. Several authors have recently drawn attention to an inherent drawback to the majority of these methods (Evans, *ANALYST*, 1927, 52, 590; Willey, *J. Soc. Chem. Ind.*, 1924, 43, 70T; Boller, *Diss.*, Zurich, 1915, quoted by Sandved, *ANALYST*, 1927, 52, 2), namely, that they do not admit of using the theoretical factor for calculating tin from the amount of oxidising agent (generally iodine) used, but rely on special factors found by reducing and titrating known amounts of tin under similar conditions. These authors share the view, which is supported by experimental evidence, that one of the most important conditions for the complete reduction of tin is that air shall be rigorously excluded from the test solution during the reduction. Inaccuracy due to the tin in the sample and in the standard not being reduced to the same extent would be at a minimum when these methods are used by skilled workers who can carry out determinations under strictly reproducible conditions, but is apt to be pronounced when determinations are only carried out occasionally, as, for example, in certain "umpire" analyses.

**LEAD AS REDUCING AGENT.**—Metallic lead would seem to offer advantages over practically every other metallic reducing agent suggested for tin, by reason of the great purity of the commercial metal, thus avoiding "blanks,"\* very often associated with metals like iron and nickel, and also because it does not introduce any coloured products into the solution to affect the delicacy of the end-point; neither does it need to be withdrawn from the solution before the titration with iodine, the end-point of which is sharp and reasonably stable (Powell, *J. Soc. Chem. Ind.*, 1918, 37, 285T). Furthermore, lead is free from the erratic behaviour in reducing properties met with in the case of zinc, which is apparently due to the free

\* The lead used in the present work gave a "blank" of 0.3 c.c. of 0.01 *N* iodine, which is of significance only in the determination of the smaller quantities of tin.

reducing surface becoming protected by a layer of spongy tin (*cf.* Sandved, *ANALYST*, 1927, 52, 2, and authors he cites).

It was, therefore, decided to use Powell's lead reduction in a modified method in which air could be rigorously excluded during the reduction and titration. As has been mentioned, several authors have suggested apparatus for this purpose, but no apparatus seemed so simple or suitable as that described by Evans (*ANALYST*, 1927, 52, 570). It consists of a conical flask of 750 c.c. capacity having a rather wide neck into which fits a rubber stopper carrying the following attachments: (a) Leading tube, from a Kipp's apparatus delivering carbon dioxide, passing down to about  $\frac{1}{2}$ -inch above the surface of the liquid which is to be reduced in the flask (it is preferable that the lower part of this tube should be bent towards the wall of the flask, so that it is well clear from the drops of iodine during the titration); (b) a small tapped funnel with its stem bent twice, so that the bulb of the funnel clears the top of the rubber stopper (to allow of the jet of a burette being inserted in the third hole); (c) a removable glass plug fitting into the third hole in the stopper.

**METHOD.**—The following method was adopted for the determination of tin: The solution to be analysed, which should contain the equivalent of 50 c.c. of concentrated hydrochloric acid, is transferred to the 750 c.c. flask, diluted to approximately 250 c.c., and a strip of stout lead foil, 12"  $\times$  1", added.\* The stopper carrying the attachments is placed in position. The tap of the funnel being open, the liquid is boiled gently for 1½ hours (at such a rate that the volume is not reduced to less than 100 c.c. during this time), a stream of carbon dioxide (which has bubbled through water and is free from air) being passed into the flask meanwhile. It is advisable that the flask should be protected from draughts during the boiling, to avoid the possibility of air being drawn into the flask. Quite a slow stream of carbon dioxide is all that is necessary, particularly as a certain volume of liquid collects in the tap funnel and helps to prevent back diffusion of air.

At the end of the specified time the flask is removed from the heat, the current of carbon dioxide being simultaneously turned full on. The tap of the funnel is now closed, and the flask removed to a cooling bath and allowed to remain under pressure of the gas from the Kipp's apparatus until quite cold. Sufficient starch indicator is poured into the funnel. The glass plug is withdrawn from the stopper and the starch solution is run into the flask, the tap of the funnel being closed before the funnel is quite empty. The jet of a burette containing iodine is now inserted into the hole formerly occupied by the glass plug (the jet must make a tight joint with the stopper, and must be long enough to project below it). The interior of the flask is now under pressure from the Kipp's apparatus, so that before iodine can be run into the flask the pressure must be released by turning off the carbon dioxide supply and opening the tap of the funnel for a moment. The titration is now proceeded with up to the starch blue end-point. For amounts of tin below 10 to 15 mgrms., 0.01 N iodine solution is used. One c.c. of 0.1 N iodine = 0.00594 grm. of tin; 1 c.c. of 0.01 N iodine = 0.000594 grm. of tin.

\* Powell used granulated lead.

**TESTS OF THE PROCESS.**—For the larger quantities of tin, known weights of metal were dissolved in warm hydrochloric acid (50 c.c. of sp. gr. 1.18), with sufficient bromine to impart a yellow colour to the final solution, which was diluted to 250 c.c. before reduction. Smaller quantities of tin were taken in the form of measured amounts of a standard solution, which were added to hydrochloric acid and water, making the conditions the same as for the larger quantities of tin. The process was carried out as described and the following results were obtained:

TABLE I.

Tin taken. Grm.	Iodine required. c.c.	Tin found. Grm.
0.2025	34.00 0.1 N	0.2020
0.1022	17.15 "	0.1019
0.0521	8.80 "	0.0523
0.0220	3.70 "	0.0220
0.0103	1.75 "	0.0104
0.0100	16.8 0.01 N	0.0100
0.0050	8.1 "	0.0048
0.0030	4.95 "	0.0029
0.0020	3.2 "	0.0019
0.0010	1.65 "	0.00096
0.0005	0.85 "	0.00051

(A blank of 0.3 c.c. of 0.01 N iodine was deducted from the total titration amount in the last six of the above experiments.)

The iodine solution used for the titration was standardised with arsenious oxide.

The time taken for the reduction is somewhat longer than is usually specified for reduction by lead; but the time cannot be shortened in this method with safety. For example, with a 40-minute period of reduction (timed after some bromine which the liquid contained had become reduced) a titration registered 0.0093 gm. of tin, instead of 0.0123 gm. which the solution actually contained. It is possible that reduction would be more rapid in a solution of greater acid concentration than that used in the present method; a more strongly acid liquid would, however, be prone to attack the rubber stopper, and a further disadvantage would be the need for diluting the reduced solution, before titrating, with oxygen-free water, since it has been noticed that the end-point is the less stable the higher the acidity of the solution, indicating a certain interaction between the lead strip and iodine in presence of much acid.

The lead reduction method for tin is generally regarded as being very little subject to interference by, at any rate, those metals commonly met with in non-ferrous analysis. Indeed, I have not seen any statements in the literature to contradict the notion that the method is suitable for the direct determination of tin in non-ferrous metals containing appreciable amounts of copper or antimony.

Prior to the experimental work described in this paper, the practice in this laboratory was to separate copper before determining tin, mainly on account of a belief that copper which would collect on the lead during the reduction process may reduce the efficiency of the reduction at the lead surface.

**THE INTERFERENCE OF COPPER.**—A noticeable effect occurs when copper is precipitated on lead from a boiling solution of hydrochloric acid containing stannic chloride. In the absence of tin in the solution, copper precipitates on the lead as a red metallic film which separates as brownish spongy masses when the amount of copper is large. In the presence of tin, there may be a momentary coating of the lead by the red film, but this rapidly turns black and becomes detached from the lead in a black flocculent form when appreciable amounts of copper and tin are present in the solution.

A series of determinations of tin was carried out by the method just described upon solutions containing known amounts of tin and copper, and the following results were obtained:

TABLE II.

	Taken.		Titration.		Found.	
	Tin.	Copper.		Iodine.	Tin.	Tin precipitated (by diff.).
	Grm.	Grm.	c.c.		Grm.	Grm.
(1)	0.2000	Nil	34.00	0.0990 <i>N</i>	0.1999	—
(2)	0.2000	0.01	33.15	„	0.1949	0.0053
(3)	0.2000	0.05	29.10	„	0.1711	0.0289
(4)	0.2000	0.10	24.90	„	0.1464	0.0536
(5)	0.2000	0.20	15.10	„	0.0888	0.1112
(6)	0.2000	0.40	1.65	„	0.0097	0.1903
(7)	Nil	0.20 less than 0.05	„	„	—	—
(8)	0.0103	Nil	17.6	0.0099 <i>N</i>	0.0103	—
(9)	0.0103	0.0010	16.9	„	0.0099	0.0004
(10)	0.0103	0.0050	12.7	„	0.0075	0.0028
(11)	0.0103	0.010	8.2	„	0.0048	0.0055

A unit weight of copper causes the loss, roughly, of half a unit weight of tin. The effect of copper in the lead reduction method for tin is, therefore, to cause the results for tin to be low in direct proportion to the amount of copper present.

In experiments (3) to (6) the major portion of the deposit flaked off the lead and became suspended in the solution in a flocculent form. After the titration, the suspended matter was filtered off and washed with water. The precipitates from experiments (3), (4) and (6) were tested qualitatively by dissolving them in concentrated nitric acid; blue solutions were produced containing heavy white precipitates which were identified as a tin hydroxide. The proportion of copper to tin in the precipitate from experiment (5) was determined as follows: It was washed from the filter with water, nitric and sulphuric acids were added, and the liquid evaporated until fumes of sulphuric acid appeared; on treatment of the residue with cold water a clear solution was produced, which remained clear and

free from deposit after being kept several hours, showing that it contained no appreciable amount of lead; the solution was electrolysed for copper, and the tin was precipitated from the copper-free solution as sulphide, which was ignited and weighed as stannic oxide. Found: Copper, 0.1700 grm. (63.63 per cent.); tin, 0.0972 grm. (36.37 per cent.). (These weights are, of course, less than those appearing for Expt. (5) in the table, owing to a part of the metals remaining adherent to the lead strip.) The deposits obtained with the smaller amounts of tin and copper (Expts. (8) to (11)) likewise showed the presence of tin when tested by dissolving the whole lead strip, carrying the deposit, in nitric acid (a clean lead strip which had not been used for a reduction showed a trace of tin in this test which was, however, qualitatively less than the amounts shown in the above tests).

It is unlikely that the precipitated metal reacted to any extent with iodine during the titration, since reasonably stable end-points were obtained, or, in view of the result of Expt. (7), that any copper in the cuprous condition remained in the solution after reduction. It is, therefore, sufficiently certain that the figures given in column 4 of Table II, which are the differences between the tin taken and that calculated from the iodine consumed in the titration, represent tin precipitated with the copper under the influence of the lead used in the reduction.

These results, plotted as a graph, show that the relationship between the amount of copper present in the original solution and the amount of tin precipitated in Expts. (1) to (5) is very nearly linear. From an extrapolation of this graph, 0.2 grm. of tin would require 0.3525 grm. of copper for its complete precipitation, thus corresponding to a precipitate containing 63.8 per cent. of copper and 36.2 per cent. of tin. These figures are closely similar to the results given above of the analysis of a specimen of one of the precipitates, and seem to suggest that the tin is precipitated with the copper as a *compound*,  $\text{Cu}_{10}\text{Sn}_3$ , which has the calculated composition: copper, 64.1 per cent.; tin, 35.9 per cent. While there is little doubt that the consistency of the results obtained indicates compound formation between the copper and the tin, it is unsafe to draw too definite a conclusion, from the evidence available, as to the chemical individuality of the substance precipitated.

THE INTERFERENCE OF ANTIMONY.—When a solution of antimony in dilute hydrochloric acid is boiled with lead, the antimony, as is well known, deposits on the lead in a non-adherent form. Experiments were carried out to ascertain whether the precipitation during the reduction of tin by lead would exercise any influence on the accuracy of the determination. Solutions containing varying amounts of tin dissolved in 50 c.c. of concentrated hydrochloric acid and oxidised with bromine, with antimony added in the form of a solution of antimonious chloride, were diluted to 200–250 c.c. The reduction with lead and titration with iodine were carried out exactly as in the standard method described for the determination of tin (Expts. (a), Table III); the precipitated antimony suspended in the solution, however, rendered the detection of the end-point of the titration



difficult. After the titration, the solution in the flask was filtered to remove the metallic precipitate, and the clear filtrate, together with the liquid used in washing the precipitate, was re-reduced, using a fresh strip of lead, and titrated as usual (Expts. (b)). The bulk of the antimony separated during the first reduction process and only a small quantity appeared on the lead during the second reduction. The following results were obtained:

TABLE III.

	Taken.		Titration.		Found. Tin. Grm.
	Tin. Grm.	Antimony. Grm.	c.c.	Iodine.	
(1) (a)	0.1057	0.5	18.7	0.1 N	0.1111
(b)			17.3	"	0.1028
(2) (a)	0.2004	0.5	34.7	"	0.2061
(b)			32.65	"	0.1939

The amounts of tin found in Expts. (b) show that co-precipitation of tin had taken place during the reduction process in Expts. (a). In spite of this, the results of Expts. (a) are seen to be high. This can only be explained by the assumption that antimony in the fine suspension reacted to some extent with iodine during the titration process. In this connection it may be noted that I have obtained distinctly high results in the determination of tin, using antimony powder which had been very finely ground in an agate mortar, and which remained largely suspended in the liquid during the titration with iodine (the end-point was not distinct); the reduction and titration were carried out in the same manner as in the method described using lead.\*

It is evident from Expts. (b) that a considerable amount of tin is removed from the solution by the precipitate of antimony. More conclusive evidence of this effect than is provided by these titration experiments has been obtained by actually determining the co-precipitated tin in the antimony precipitate. For this purpose the precipitate of antimony obtained by carrying out the usual lead reduction process on solutions containing tin and antimony was collected by filtration (most of what remained adherent to the lead strip was recovered by spreading the strip diagonally upon a glazed tile, rubbing off the deposit with a rubber-tipped rod, and rinsing it on to the filter), and well washed with hot dilute hydrochloric acid. The deposit was dissolved by treating the filter with dilute hydrochloric acid and bromine. The clear solution, to which was added 1 grm. of citric acid, was boiled to remove excess of bromine, neutralised with ammonia and 2 per cent. (by volume) of hydrochloric acid added. To the cold solution (volume 200 c.c.) cupferron was added, when a white precipitate was produced, showing the presence of tin, since interfering metals were absent and quinquivalent antimony gives no precipitate with cupferron under these conditions. The precipitate was filtered

\* Incidentally, when somewhat coarser antimony (over half of which passed through a 90-mesh sieve) was used in the reduction distinctly low results were obtained.

off, and washed with a dilute solution of cupferron; the filter was ashed, ignited, and the residual tin oxide weighed. The following results were obtained:

TABLE IV.

Taken.		Found.	
Tin. Grm.	Antimony. Grm.	Weight of tin oxide. Grm.	Tin recovered. Grm.
Nil	0.2	(0.0004)	—
0.100	0.2	0.0070	0.0056
0.200	0.2	0.0122	0.0096

Wide variations from the theoretical results are, therefore, obtained in the determination of tin by the lead reduction process in presence of appreciable amounts of antimony. It is clear that this is due to errors caused by (a) reaction of iodine, during the titration, with the precipitated antimony, (b) co-precipitation of some tin with the antimony. These errors, while acting in opposite directions, do not necessarily compensate one another.

THE EFFECT OF ANTIMONY AND COPPER TOGETHER.—Metallic copper is known to form antimonides readily, the compound  $\text{Cu}_2\text{Sb}$  having been found to be the chief constituent of antimony Reinsch films (Evans, ANALYST, 1923, 53, 1). The extent to which tin is carried down with either copper or antimony in the lead reduction process might be expected to be less if copper and antimony were present together than if they were present separately, as in the foregoing experiments, owing to the possibility of copper combining with the antimony in preference to tin. This was found to be the case in an experiment in which copper (0.51 grm.) and antimony (0.5 grm.) were added (as chlorides), in approximately the proportions in  $\text{Cu}_2\text{Sb}$ , to a solution containing stannic tin (0.2530 grm.); after reduction by the usual process, 0.2414 grm. tin was found by titration. This result is low, but is very much higher than would have been obtained in absence of the antimony.

Many commercial alloys in which tin is likely to be determined by the lead reduction process, such as tin base bearing metals, contain several units per cent. of antimony and copper, and the antimony is usually present in greater proportion than the copper. The interference of these metals with the tin determination in these cases is not likely to be very noticeable, although it may account for some of the difficulties met with in obtaining a satisfactory analysis of these alloys.

It may be concluded from the above work that where copper and antimony are present it is, in general, necessary to effect a preliminary separation of the tin if results are to be obtained of the accuracy of which the lead reduction method, in its present form, is capable.

## The Electrolytic Separation of Lead and Antimony, and its Application to the Determination of Lead in Tartar Emetic.

BY ELLA M. COLLIN, B.Sc., A.I.C., AND  
HENRY J. S. SAND, D.Sc., Ph.D., F.I.C.

DURING the course of some experimental work on the analysis of lead bullion by internal electrolysis (E. M. Collin, *ANALYST*, 1930, 314) it was found that antimony may be deposited quantitatively from a solution containing the metal in the antimonious state, when only the limited potential supplied by a lead anode is available. On the other hand, antimonic antimony is not deposited at all under these conditions. It was previously known that, under conditions in which the deposition potential is not limited, antimony deposited from a solution containing antimonious chloride is less compact than that obtained from the antimonic compound (Lassieur, *Electroanalyse Rapide*, Paris, 1927, p. 87), and that the metal in the pentavalent state is not deposited at all from fluoride solutions in the presence of potassium dichromate (*ibid.*, p. 95). These facts form the starting point of the present investigation.

The deposition potentials of trivalent antimony, of pentavalent antimony, and of lead in acid tartrate solutions were compared, the following being made up:

### I. In TARTARIC ACID.

- A. 0.25 gram. of tartar emetic, 5 grms. of tartaric acid, 3 grms. of sodium tartrate, and about 200 c.c. of water.
- B. 0.25 gram. of potassium antimonate, 5 grms. of tartaric acid, 3 grms. of sodium tartrate, and about 200 c.c. of water.
- C. 0.25 gram. of lead nitrate, 5 grms. of tartaric acid, 3 grms. of sodium tartrate and about 200 c.c. of water.

The deposition potentials were measured against a tenth normal quinhydrone electrode. In A, antimony was deposited when the potential was raised to 0.9 volt, in B, no antimony was deposited even when the potential was raised to 1.1 volt, and in C, the deposition potential was 0.98 volt.

### II. IN NITRIC AND TARTARIC ACID SOLUTIONS CONTAINING CHLORIDES.

- D. 10 c.c. of concentrated nitric acid, 1 gram. of tartaric acid, 2 c.c. of 2 *N* hydrochloric acid, 0.5 gram. of hydroxylamine hydrochloride, 0.25 gram. of tartar emetic, and about 200 c.c. water.
- E. As D, but 0.25 gram. of potassium antimonate instead of the tartar emetic.

F. 5 c.c. concentrated nitric acid, 2 grms. of tartaric acid, 2 c.c. of 2 *N* hydrochloric acid, 1 gm. of hydroxylamine hydrochloride, and 0.25 gm. of lead nitrate.

In D, antimony was deposited at a potential of 0.75 volt, in E no antimony was deposited when the potential was raised to 1.1 volt, and in F, the deposition potential was 0.92 volt.

These experiments furnish a sufficient explanation of what has been stated concerning the deposition of antimony in the internal electrolysis apparatus.

The deposition of antimony from alkaline solutions was next tried, and it was found that, whereas antimony in a spongy form may be deposited from an antimonite solution, none is precipitated even by high current densities when an antimonate solution is employed. Lead, on the other hand, may readily be deposited as metal in a satisfactory form from alkaline solutions (Sand, *J. Chem. Soc.*, 1907, 91, 397).

On these observations a method of separating lead from antimony was based, the principle of which is the following:—The antimony is oxidised to the antimonie state by iodine in the presence of bicarbonate. Tartaric acid is then added, and the solution made strongly alkaline with potassium hydroxide. It is then analysed by means of a current which may be derived either from an external source or from internal electrolysis.

ORDINARY ELECTROLYSIS.—Tests were first made in which an externally generated current of about 3 amperes was employed, the electrodes previously described being used (Sand, *ANALYST*, 1929, 54, 275). Five grms. of "lead-free" tartar emetic were dissolved in water, 3 grms. of potassium bicarbonate added, and the antimony oxidised with a solution of iodine in potassium iodide, after which about 4 grms. of potassium hydroxide and 2 to 3 grms. of tartaric acid were added. The solution, after dilution to a volume of about 300 to 350 c.c., was electrolysed cold. The platinum cathode was previously coated with copper and the anode rotated. There was a small deposit of lead on the cathode, the presence of which was confirmed by a chemical test. The sample of tartar emetic was tested chemically and found to contain lead. A standard solution of lead acetate was made, and known amounts added to a solution of tartar emetic prepared as described above and electrolysed. A deduction was made from the weight of the deposit to allow for the lead in the tartar emetic unless this had been removed previously by electrolysis. The following results were obtained:

Lead added. Grm.	Lead found. Grm.
0.0033	0.0031
0.0024	0.0022
0.0016	0.0017
0.0011	0.0011
0.0023	0.0022
0.0016	0.0016

**INTERNAL ELECTROLYSIS.**—The solutions for electrolysis were prepared as described above and electrolysed in the internal electrolysis apparatus (Sand, ANALYST, 1930, 309), using zinc anodes and a 10 per cent. solution of potassium cyanide in the anode compartments. Zinc nitrate as the anolyte did not give a sufficient e.m.f. to effect the deposition of the lead. Results obtained were as follows:

Lead added. Grm.	Lead found. Grm.
0.0034	0.0033
0.0022	0.0019
0.0028	0.0028
0.0016	0.0016
0.0011	0.0011
0.0033	0.0033
0.0033	0.0031
0.0033	0.0032

The time of electrolysis was in each case 15 to 20 minutes.

**DETERMINATION OF LEAD IN TARTAR EMETIC.**—In the following experiments samples of tartar emetic were analysed for lead both by ordinary and internal electrolysis, and the results were checked by a chemical method, namely, the precipitation of the lead as sulphide and weighing it as lead sulphate.

No.	Ordinary Electrolysis.		Internal Electrolysis.		Chemical.	
	Amt. taken Grams.	Lead Per Cent.	Amt. taken Grams.	Lead Per Cent.	Amt. taken Grams.	Lead Per Cent.
1	2	0.096	3	0.076	2	0.096
2	3	0.080	2	0.075	2	0.092
	3	0.083				
3	3	0.09	3	0.09	2	0.09
	2	0.11	2	0.085	*2	0.097
	2	0.10	2	0.075		
4	*2	0.021	5	0.020	5	0.028
					*2	0.026
					*5	0.021
5	10	0.007	10	0.009	10	0.005
	*10	0.0083			*5	0.0076

The results we have obtained are satisfactory, both when a current derived from an external source and when one obtained by internal electrolysis was employed. We have no hesitation in recommending the first-named method; before, however, definitely advocating the use of the second for analysis, we should like to have a further opportunity of investigating the effect on the copper cathode, of cyanide, which may find its way into the catholyte from the anode compartment.

In addition to the gravimetric method, three of the samples were tested by a colorimetric method, in which the lead is precipitated as chromate and determined

\* Colorimetric determination.

with diphenylcarbazide as indicator (B. Jones, ANALYST, 1930, 55, 318). The sample was dissolved in water, the solution made alkaline with potassium hydroxide, and the lead precipitated as sulphide, the subsequent procedure being as described in the paper quoted. In addition, the deposits obtained electrolytically from the two samples of low lead content were dissolved and tested by the same colorimetric method. The copper used for plating the cathode did not interfere.

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## The Testing of Admiralty Disinfectant Fluid.\*

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**INTRODUCTORY.**—The Admiralty Disinfectant Fluid is of the type commonly known as a "coal-tar disinfectant," and in modern practice the raw material is obtained almost exclusively from coke-oven, producer-gas, and blast furnace plants. The active ingredients derived from the tar distillate fraction are phenoloids; phenol itself is, or should be, absent. The germicidal action of the fluid is due to the homologues of phenol, the cresols, xlenols, and still higher homologues.<sup>1</sup>

These compounds, with increasing molecular weight, exhibit greater germicidal power and decreased toxicity. The higher homologues are relatively insoluble in water, and, therefore, to render them available for use as disinfectants, they have to be emulsified. Soaps are largely employed for this purpose, but are inadmissible for use in the Admiralty Disinfectant Fluid, which is required to be miscible with sea-water in all proportions. The germicidal power of such a disinfectant is related not only to its chemical composition, but directly also to its physical condition, as expressed by the fineness and stability of the emulsion.

**SAMPLING.**—An original unopened container is forwarded for examination, unless the capacity is greater than one gallon, in which case a sample of about 500 c.c. is withdrawn after thorough mixing.

**PHYSICAL CONDITION.**—The entire sample is transferred to a narrow-mouthed stoppered bottle of capacity slightly in excess of the volume received for examination. It is mixed by pouring the whole, five times backwards and forwards, from the bottle into the container, with the aid of a funnel 15 cm. in diameter, and finally into the bottle.

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Quantities of 250 c.c. and 100 c.c. are placed in stoppered cylinders of 1 litre capacity. Each is made up to 1 litre with artificial sea-water, and thoroughly mixed. The artificial sea-water is prepared by dissolving 27 grms. of sodium chloride (A.R.) and 5 grms. of magnesium sulphate (A.R.) in sterile distilled water, and making up to 1 litre. The cylinders are allowed to stand in the dark for 6 hours, at the end of which time visual examination is made of these dilutions (25 and 10 per cent.); and after 24 hours, the 1 per cent. dilution, prepared for estimation of "phenol coefficient" (*vide infra*), is similarly examined to determine whether the sample is *miscible with sea-water in all proportions*.

The stoppered bottle, with the remainder of the sample, is allowed to stand in the dark for 7 days. It is then inspected to ascertain whether it is *entirely free from sediment and does not separate out or precipitate, also whether it has any objectionable or disagreeable smell*.

If the sample has been received, as is usual, in its original container, the latter is examined for evidence of *corrosive action on metals*.

**GERMICIDAL VALUE.**—It must be recognised that the germicidal value expressed as "phenol coefficient" has a very limited interpretation in actual surgical and sanitary practice. The test consists in comparing the lethal power of the product under consideration with that of pure phenol. In most methods, whether the original<sup>2</sup> or the improved Rideal-Walker,<sup>3</sup> the U.S.A. Hygienic Laboratory,<sup>4</sup> or the Chick-Martin,<sup>5</sup> the basis of comparison is the inhibitory effect on a certain standard test organism, namely *Bacillus typhosus*.

The behaviour of a germicide with *B. typhosus* is no criterion of its action against other more common pathogenic organisms. Before it could be laid down that such and such dilutions should be used for, say, disinfection of drains, utensils, clothing, hands, it would be necessary to test the product against the actual bacteria usually found in these situations. Antiseptics for use on the skin and mucous membrane should be examined in the presence of pyogenic organisms such as *Staph. aureus* and *Strep. haemolyticus*. If a disinfectant is recommended for general use, the manufacturers should be able to guarantee that the dilutions specified will kill all the non-sporing organisms which cause diseases of an epidemiological nature.<sup>6</sup> Again, the optimum temperatures for pathogenic organisms differ from what is laid down in the laboratory test. The organic matter with which they may be associated is a very variable quantity; the time of exposure and the opportunities for penetration may not coincide with the artificial conditions of phenol coefficient technique. For these reasons it has been stated that a phenol coefficient test is unfair and has no value in practical medicine. These objections are valid only if an estimation of exact germicidal effect is desired. This information was never expected to be obtained from such a process as the Rideal-Walker or its modifications, the aim being confined to a numerical comparison of disinfectants with a standard.<sup>7</sup> Its applications are limited, but it is a definite means of ascertaining the comparative value of disinfectant fluids; it gives also at least a rough indication of their practical value.

The determination of the phenol coefficient by any method is entirely empirical, and the strictest attention to every detail is essential to secure concordant

results. Unless every step is carefully standardised, different operators may report entirely different values for the same sample.

Two disinfectant fluids, which may be designated A and B, were divided into two series of identical samples, and were sent to thirteen laboratories recognised as undertaking the examination of such fluids. The phenol coefficient of these samples, as determined by the Rideal-Walker technique, was reported by the various laboratories as follows:<sup>8</sup>

Laboratory	1.	2.	3.	4.	5.	6.	7.
Sample A.	8.95	5.5	5.5	6.0	6.0	6.25	7.0
Sample B.	11.8	6.0	14.4	9.0	12.0	9.0	14.0
Laboratory	8.	9.	10.	11.	12.	13.	
Sample A.	10.0	10.5	10.0	11.0	11.5	11.2	
Sample B.	11.0	12.7	14.0	17.0	16.4	16.4	

The most cursory examination reveals the enormous differences in these figures, which are quoted to show the necessity for the extreme, and perhaps tiresome, detail with which the Admiralty method is described in the subsequent pages.

In outline, the method consists in preparing a 1 per cent. dilution of the fluid with artificial sea-water, allowing this to stand for 24 hours; and then determining its action in presence of organic matter, against a particular strain of *B. typhosus*, as compared with pure phenol.

Some of the causes of the great variation in the results of phenol coefficient determinations are common to all methods, and are referred to in discussing certain details of the Admiralty method under the headings:

**THE TEST ORGANISM AND ITS PRELIMINARY SUBCULTURE.**—The same species of organism must be used in all series of tests if any uniformity of results is to be looked for, and also, for comparative work, the ideal is to have a culture of standard strength and resistance. Discrepancies up to 300 per cent. may occur if different species are taken, and there may be variation even between strains of the same species. *B. typhosus*, adopted originally by Rideal and Walker, is still recognised as the best choice. It is more constant in behaviour than most organisms, produces an easily recognised turbidity in broth as evidence of growth, and can be readily counted or agglutinated in the endeavour to arrive at a standard strength. The use of a set type such as *B. typhosus*, Rawling's (National Collection of Type Cultures) further diminishes the opportunity for variation. This is a remarkably constant strain, and has retained its virulence, antigenic power, and agglutinability for years. *B. coli*, recommended in the *Lancet* method<sup>9</sup> on account of its non-pathogenicity, is an organism whose strains vary considerably in resistance.

In order that the culture shall be of optimum vitality on the day of the test it is important to have a recent growth in broth—24 hours at 37° C. The resistance, even when subculturing from the same stock, may vary from day to day<sup>10</sup>; forty-eight hour cultures generally are more resistant. The number of



times the organisms are subcultured before use will considerably affect the result. A coefficient may vary according to this number, and tend to be higher the younger the generation.<sup>7</sup> Conditions can, therefore, be further standardised by subculturing daily in a set medium for a set period, say 7 days, starting from a stock agar of a certain age, *e.g.* one month, that has been stored at room temperature.

In this laboratory the present routine for preparing the test cultures is as follows:—The organism is *B. typhosus*, Rawling's strain (N.C.T.C.). Monthly subcultures are grown on nutrient agar made with the same broth basis as is specified for the test. Dry slopes are chosen, as moist media favour the change from smooth to rough growth. Stocks are maintained at room temperature. It is assured that the strain reacts constantly to high titre serum and retains smoothness and motility. Subcultures in broth (prepared as detailed) are started afresh every week. Taking a month old stock agar and plating out overnight at 37° C., a smooth colony of 1 mm. diameter is lifted and sown whole into the first broth. Re-inoculations are made every 24 hours with the standard 4 mm. loop (*vide infra*). Tubes are of uniform size and contain 5 c.c. of broth. Suitable strength is assured by matching between stock tubes (1000 millions and 2000 millions per c.c.), preserved from previous satisfactory tests, sterilised, counted and sealed. If turbidity is insufficient, a heavier inoculum is prepared ready for the next 24 hours. The series is maintained at 37° C., and the day before the test, transfers are made to four or more such broths, according to the amount of culture required. Satisfactory readings are obtained with from 3 days to a fortnight's subculturing, after which time there is a tendency for the rough phase to supervene. For the sake of uniformity the routine period is made 7 days. The object is to stabilise the resistance of the test organism. On plating out for a new series of broths a pure smooth colony is chosen—moist, translucent, domed, free from radiation or granular appearance, and giving uniform turbidity in the fluid medium. The organism is then at the height of its virulence and antigenic power; its strength is at a definite level—the optimum. It can likewise be accredited with possessing some definite degree of susceptibility to antiseptics. On the other hand, there are countless gradations of the partially rough variants, with corresponding uncertainty in their reactions down to the wholly rough type—dry, crenated, granular, opaque, precipitating in broth, auto-agglutinable and weak in antigenic power. Similarly, their resistance to antiseptics must vary. If the series is always initiated from a smooth strain of a definite age; is subcultured in standard broths over a definite period; and gives a standard opacity, there should be more likelihood of standard resistance in the test culture.

**PRESENCE OF ORGANIC MATTER AND ITS NATURE.**—In order that the conditions under which the disinfectant is tested for phenol-coefficient shall simulate what occurs in practice, organic matter is added. From time to time various authorities have favoured admixture of blood serum, dead cultures, urine, faeces, peptone, gelatin, egg albumin, pus, mucin, casein, and starch. It is important to prove the efficiency of the sample in the presence of some such material; but it is essential that it shall be sterile and of known definite composition. The difficulty of ensuring sterility of blood serum as such, makes its routine use hardly practical. The presence of urine, faeces or pus would, perhaps, represent natural conditions best, but their organic content is widely inconstant. Where every endeavour is being made to reduce variable factors something more reliable is required. As

it is impossible to obtain morbid secretions and excretions of uniform character and composition, combinations of certain types and strengths of standard material are used. Thus the interaction between the organic matter and the disinfectant is more under control.

In the Hygienic Laboratory (U.S.A.) method,<sup>2</sup> 3 per cent. of organic matter, in the form of a peptone-gelatin combination, is mixed with the disinfectant dilutions. The materials are carefully standardised and satisfactory results are claimed. Carbohydrate plus protein makes a still more natural combination. Adapting from the work of Sommerville and Walker,<sup>11</sup> particulate matter, which will introduce the influence of adsorption, is incorporated in the Admiralty technique in the form of a suspension of rice starch. This substance can be ground very fine. Compared with the other commonly occurring starches the rice granule is one of the smallest ( $3-7\mu$ ); also it is more uniform in size.<sup>12</sup> Protein, in the form of gelatin, is introduced, but only a small percentage can be added, on account of its physical condition at the temperature of the test.

The gelatin-starch mixture is prepared in the following manner: Five grms. of gelatin are dissolved in one litre of artificial sea-water; 0.5 gm. of specially prepared rice starch is added to 100 c.c. of the stock gelatin solution immediately before the test. The rice starch is prepared by drying it at  $100^{\circ}\text{C}$ ., then passing it through a 60-mesh to the inch sieve into a Petri dish, and finally sterilising it in the hot air oven at  $130^{\circ}\text{C}$ . for 4 hours. This starch is kept in the Petri dish in the cold oven until required. The method of sterilising starch by treatment with alcohol or chloroform in alcohol and then drying in an oven has not proved satisfactory.

Experiments show that it is unnecessary to add the organic matter to the carbolic dilutions. These are aqueous solutions; therefore, the same strength of phenol is present in every ultimate portion, and admixture of particulate matter will not noticeably lessen the amount of germicide which comes in contact with the bacteria. Addition of such a substance to disinfectant emulsions affects the germicidal value to a much greater extent. Here the physical state being that of an emulsion, all the active ingredient is in the form of microscopic globules moving in the inert watery diluent. Adsorption of the disinfectant globules by the organic particles will reduce the number available to act on the bacteria.

**DILUENT.**—As the uses to which disinfectant fluid is put in the Royal Navy are generally associated with the presence of sea-water, a similar medium is prescribed in the Admiralty test. It is used as the diluent for both disinfectant and organic matter. The composition of the artificial sea-water has been given on p. 94.

**THE DISINFECTANT EMULSION.**—The main factors governing the bactericidal powers of an emulsified disinfectant of the type under discussion are (a) the nature and amount of tar acids, (b) the perfection of the emulsion. These products are physico-chemical in their action,<sup>13</sup> and have marked adsorptive properties, whereby the antiseptic constituent concentrates round the bacterial protoplasm. The

degree of homogeneity has a considerable effect upon the activity. An irregular emulsion tends to separate, and will give different values with different portions of the one sample. This de-emulsification is accelerated in the presence of an electrolyte such as sea-water. The amount of shaking employed in preparing the test dilutions is a marked source of variation. A product heavily charged with tar acids, in order to enhance its potency, may yield an unstable emulsion. If sufficiently vigorous shaking is given, the value is artificially improved; but if the dilution stands for some hours before the test is made, quite a different result is obtained.<sup>8</sup> Therefore, even in the minor matter of shaking, there should be a standard method. A good emulsion, with normal shaking, will retain a uniform homogeneity and coefficient value.

**THE PHENOL.**—Carbolic acid lends itself admirably as the coefficient denominator. It is obtainable pure, and remains stable if stored under proper conditions. Leroux<sup>14</sup> has shown that the best test for determination of purity of phenol is by means of the melting point, which should be  $40.85^{\circ}\text{C}.$ \*; the presence of 0.2 per cent. of water lowers the melting point to  $40.10^{\circ}\text{C}.$  Cresols cause a reduction in this temperature of half a degree centigrade per cent. of admixture.<sup>15</sup> The Admiralty method prescribes Calvert's No. 1 carbolic, but it is proposed to alter this to "pure carbolic acid having a crystallising point of  $40.5^{\circ}\text{C}.$ "

The accuracy of the phenol coefficient test is fundamentally dependent upon the reliability of standard solutions prepared from the phenol. The usual practice is to keep a stock 5 per cent. solution by weight, from which other dilutions are freshly made for each test as required. The routine followed in this laboratory is to keep stock quantities of all the test dilutions. The dry crystals are weighed in a stoppered weighing bottle, dissolved in sterile distilled water, and made up to the required volume. In order to obtain suitably close dilutions, and to have them arranged in mathematical order, figures are chosen which give a geometrical progression of 4 per cent. on either side of 1 in 100, *i.e.* 1 in 88.90, 92.45, 96.15, 100, 104, 108.16, 112.48, etc. Each successive denominator shows an increment of  $1/25$ . Five hundred c.c. quantities are made up and stored in amber-coloured bottles with the original stoppers, in a cool, dark cupboard. Such dilutions remain unchanged for at least a year. Five c.c. portions of the required strengths are taken for each test.

**PROPORTION OF CULTURE TO DISINFECTANT.**—The employment of various sized drops, spoonfuls, and other measures in different modifications of the phenol coefficient test must mean variation in the number of organisms introduced into the tubes. Discordant results are inevitable if some workers use, say, "0.2 c.c.," and others "5 drops" of varying size<sup>4,8</sup>. If tests are to be comparative, the dilution tubes must always be inseminated with a definite quantity of culture accurately measured by graduated pipettes. The larger the dose of culture, the greater is the effect of mass resistance, and the stronger will be the dilution of

\* Stratton and Partington (*Phil. Mag.*, 1922, 43, 411) found the melting point of pure phenol to be  $41.5^{\circ}\text{C}.$

emulsified disinfectant required to kill in standard time. Its coefficient will, therefore, be lower.

In the U.S.A. Hygienic Laboratory method<sup>4</sup> a 0.1 c.c. dose is used, measured by pipettes graduated in tenths, while 0.5 c.c. quantities are recommended by Rideal and Walker,<sup>3</sup> to be distributed by means of a dropping pipette calibrated to deliver this amount. Even with the most careful measurement, the inoculum may vary in a series of tests unless the culture is always of the same strength. In this discussion an account is given, under the heading "Test Organism," of the steps taken in an endeavour to arrive at a standard resistance. The resulting culture is somewhat susceptible, but at the dosage employed (0.25 c.c.) it works very satisfactorily, also within the limited range of dilutions chosen. The volume is delivered by means of a Cole's automatic pipette.

**AMOUNT OF INOCULUM TO BROTHS.**—As the operation must be completed within a scheduled time, the platinum-loop method is used to transfer portions of the mixture of culture and disinfectant to the final broths. The quantity thus carried over is small enough to ensure a dilution in the broth which will prevent any further inhibitory action on surviving bacteria. If these quantities vary, a positive growth may occur after exposure to slightly stronger solutions of disinfectant and a lower coefficient will be obtained. Hence the recommendation for a standard-sized loop in standard gauge wire, the transfer to be carried out in standard manner by immersing the loop at a definite angle to a definite depth. Here the U.S.A. Hygienic Laboratory technique is adopted as most reliable, except that, as far as possible, the same platinum loop is used throughout the test, instead of having a number to choose from. This lessens the chance of variation in size of inoculum. Platinum wire, gauge, 0.015 mm. B.W.G. No. 2, is employed. A perfectly circular loop, 4 mm. inside diameter, is fashioned on round-nosed pliers leaving a shank, 3.5 cm. long, to fit into the holder. A wooden end prevents the rod from getting too hot for the hand with the repeated sterilisations necessary during the test. The loop is set accurately at an angle of 45° with the shank. The loopfuls are taken from the dilution tubes without moving these from their upright position in the water-bath. The rod is held vertical, and the wire is immersed to the same depth and lifted with the same quick motion for each tube. Similar stereotyped movements are used when inoculating the broths.

**MEDIA.**—The constitution of the subculture broth and its pH value are most important considerations. The medium must be at optimum reaction for the test organism; otherwise growth may be inhibited after transplanting. For example, if a transplantation is made from a test dilution which is just under the killing strength of the disinfectant, the inhibitory action of the medium may be sufficient to prevent growth and give a false result.<sup>4</sup>

Broth made from fresh meat encourages growth better than meat extract broth, and may give a positive finding from a stronger dilution tube. The brand of peptone and the percentage of this ingredient also affect the result. Broth with a higher content of peptone gives higher coefficients.

Commercial peptones of different brands vary greatly in composition, and this lack of uniformity causes variations in nutrient value of broths. The coefficient returned for identical samples of a disinfectant may vary as much as 100 per cent. in different laboratories from this one factor. Most authorities recommend that the broth should be standardised to  $pH$  7.6. At this optimum value constant findings are obtained, whereas at higher or lower reactions the coefficient varies indefinitely.<sup>16</sup> Even into the preparation of broth a personal element may enter and affect results; workers may differ in their readings of a colour reaction in titration.

A successful technique will be one where these opportunities for variation are reduced to a minimum; the broth must be prepared according to one standard method, in which the same approved peptone is always used and the medium is brought to the optimum  $pH$  for the organism concerned. In this laboratory the method of preparing the broth is that described by McIntosh and Smart<sup>17</sup>; Armour's peptone is used.

**TIME GIVEN FOR THE TEST.**—Where transplantation from the seeded tubes is repeated every  $2\frac{1}{2}$  minutes ( $2\frac{1}{2}$  minutes, 5,  $7\frac{1}{2}$ , . . . . . 15 minutes—Rideal-Walker), a killing level may be found at more than one of these time periods. Either could be used for the coefficient reading, and the figures might be different. Also, the actual time taken for killing would not be indicated by the number reported. By having one set time for contact, the same for all dilutions, instead of a series of  $2\frac{1}{2}$  minutes' intervals, the technique is simplified, and more samples can be tested at once. The finding indicates that disinfection has been obtained after a known interval of action. Rideal later recommended such a variation of the test.<sup>18</sup> Some cultures, being more resistant, are less affected in the first few minutes of contact with a germicide. Others are more susceptible, and suffer most in the early stage of the test, after which the action of the antiseptic gradually decreases. Transplanting at a period about midway between the Rideal-Walker limits offers a satisfactory standard. Using such a method of time allowance, different workers are more likely to get similar results. The test thus modified is more suitable for commercial use and for inclusion in a contract specification.<sup>7</sup>

**TEMPERATURE OF EXPERIMENT.**—In general, the higher the temperature the greater the velocity of disinfection and the stronger the apparent germicidal power.<sup>19</sup> For example, phenol kills *B. typhosus* in  $2\frac{1}{2}$  minutes when the dilution is 1/80 and the temperature  $15^{\circ}C$ .; at a temperature of  $30^{\circ}C$ . a dilution of 1/120 has the same effect. Series of tests carried out at dissimilar temperatures cannot be accurately compared. To minimise error, the technique must be so adapted as to ensure the smallest possible range. For ordinary use the disinfectants under consideration are generally required to work at about room temperature, and a test carried out at this level is, therefore, practical, and is suitable for most climates. Rideal and Walker allow a range  $15$  to  $18^{\circ}C$ ., whilst the U.S.A. Hygienic Laboratory specifies  $20^{\circ}C$ . The Admiralty schedule prescribes  $18^{\circ}$  to  $20^{\circ}C$ ., and in this laboratory the water-bath is kept as nearly as possible at  $19^{\circ}C$ .

**METHOD.**—*Apparatus required.*—Suitable wooden racks for preliminary handling of dilution tubes.

The following glassware, sterilised in the hot air oven for  $\frac{1}{2}$  hour at  $150^{\circ}\text{C.}$ :—A wide-mouthed specimen jar (e.g.  $3''\times 2''$ ) to contain culture; glass cap and funnel with cotton-wool filter. Requisite number of dilution tubes ( $3''\times 1''$ ) cotton-wool plugged. Test tubes ( $6''\times \frac{3}{8}''$ ) to hold convenient portions of 1 per cent. sample. One c.c. graduated pipettes for disinfectant dilution. Ten c.c. pipettes, marked at 4.4 and 8.8, for measuring organic matter. Cole's automatic pipette in a suitable wide test tube.

*Water Bath.*—A water-bath with electric heating is used. It is fairly shallow, to facilitate the manipulation of the short tubes employed, and of a size capable of taking three metal racks of 24 tubes apiece. Thus the dilutions for four samples and a control are accommodated, in addition to receptacles for culture and 1 per cent. samples, e.g. five rows of seven dilutions. As a rule, fewer dilution tubes suffice, it being known within what limits the critical change from "killing" to "growing" must occur.

*Preliminary.*—Twenty-four hours before the test is to be made the sample dilution is prepared. A stoppered graduated 1000 c.c. cylinder, of not greater diameter than 7 cm., is filled to about the 980 c.c. mark with sterile artificial sea-water. A pipette graduated to hold 10 c.c.\* is filled above the mark, wiped clean outside, and run down to the mark. With the nose of the pipette in the sea-water it is allowed to discharge, and thereafter the pipette is rinsed out three times in the clear portion of the liquid. The whole is made up to 1000 c.c. with artificial sea-water. The cylinder is stoppered, and the contained liquid is thoroughly mixed by inverting, with a corkscrew motion, 100 times. It is now allowed to stand undisturbed at room temperature and away from light for twenty-four hours. Any separation is noted before using for coefficient determination.

At the same time as the sample dilution is prepared, 5 c.c. quantities of the phenol dilutions are pipetted, with a standard pipette, from the stock bottles, into appropriately marked tubes.

Other matters which receive attention the day before the test are:—The test culture (*q.v.*). The last twenty-four hour subculture is put up in a number of broths (4 or 5) sufficient for inoculating the total number of dilution tubes.

The provision of the requisite number of final broth tubes appropriately marked to tally with the individual dilution tubes. All broth is prepared according to the method referred to under "Test Organism" (*q.v.*).

The 0.5 per cent. gelatin in artificial sea-water and the starch for admixture prepared as already described.

**THE TEST.**—The first care is to bring the bath to a temperature of  $19^{\circ}\text{C.}$  Thenceforward throughout the manipulation this temperature is maintained by adjustment of the thermostat.

The following articles are then placed in the bath in order that they may attain the standard temperature:—The row of phenol dilutions transferred to one of the racks; test tubes for ready use portions of sample dilution—pipetted from the middle of the 1000 c.c. cylinder; flask of gelatin-starch mixture; jar containing filtered culture (the broths having been poured into the funnel and the apparatus covered with its sterile cap).

\* Those made for us have a sand-blasted band above and below the mark, so as to be readily distinguishable from the ordinary type.

The short test tubes for disinfectant dilutions are set out in their wooden racks, and each receives 4.4 c.c. of the organic suspension; care is taken to pipette from the body of the mixture and to avoid the heavier material at the bottom. Each tube is transferred at once to its appropriate rack in the water-bath.

A minimum coefficient of 8 being demanded by the contract specification, the strength of the phenol and of disinfectant sample can be so arranged that this reading will occur within the limits of the tubes—*e.g.* of phenol, a sequence of dilutions above and below 1 in 100, and of sample a sequence above and below 1 in 800. Were it desired to determine the coefficient of a fluid, the value of which was unknown, it would be necessary to make preliminary tests in coarser dilutions. The fluids considered here, however, are contract specimens; therefore, the end-point should occur within a limited number of tubes.

A suitable block of dilutions is prepared from the following table, *e.g.* 1/1200 to 1/589:

Starch and gelatin.	1 per cent. sample.	Total mixture.	Final dilutions.
4.4	0.1	4.5	1 in 4500
4.4	0.15	4.55	3033
4.4	0.2	4.6	2300
4.4	0.25	4.65	1860
4.4	0.3	4.7	1567
4.4	0.35	4.75	1357
4.4	0.4	4.8	1200
4.4	0.45	4.85	1078
4.4	0.52	4.92	946
4.4	0.6	5.0	833
4.4	0.7	5.1	729
4.4	0.8	5.2	650
4.4	0.9	5.3	589
4.4	1.0	5.4	540

The final strengths (column 4) are in rough geometrical progression, corresponding each time to three successive dilutions of 4 per cent.\*

As each final dilution is completed the tube is re-plugged, shaken, and replaced in the water-bath.

The water-bath is now ready with its racks of dilution tubes of both samples and standard phenols.

The exact time is now noted, and at regular intervals (a stop-watch is used), say, 15 or 20 seconds, 0.25 c.c. of filtered culture is pipetted into each tube in succession. These tubes must now be shaken again so that the organisms will be evenly distributed. It is convenient for the assistant to lift the tubes out of the rack one at a time, remove the wool plug, and hold it resting upright on the carbolised towel ready for the culture. Each tube is then shaken and returned to its position in the rack. Allowing 15 seconds per tube, up to three dozen tubes can be treated, well within the prescribed ten minutes' action period.

\* Here all the tubes carry an excess of organic matter, and it might be argued that the weaker dilutions of sample have to act against an unfair load. This objection has been met by mixing the sample dilution with organic suspension in bulk; and distributing in arithmetical series by means of burettes; but this process has proved too lengthy for routine work. The method described above has been found satisfactory in practice. Comparison of results shows no appreciable difference in coefficient findings.

At exactly 10 minutes from the time first noted, and at the same number of seconds' interval as before, an inoculum is taken from each tube in the same order and planted into broth. The assistant hands each broth tube to the operator and removes the corresponding dilution tube from the water-bath as soon as the inoculum has been taken. For this purpose the platinum needle, with its standard 4 m.m. loop set at 45°, is plunged vertically into the solution to a definite depth, and then lifted with a quick motion—uniform throughout, in order always to produce a loopful of the same size. The broth tube has been taken in the left hand and the plug removed with the right little finger. The tube mouth is flamed, and the loop is carefully inserted so as not to foul the sides. After 15 seconds the loop is plunged into the broth, withdrawn, and flamed. The re-plugged tube is then handed to the assistant in exchange for the next, and the manoeuvre is repeated similarly for the whole series.

On completion, the broth tubes, now replaced in order in their wooden racks, are incubated at 37° C. for 48 hours. An end-point between killing (clear) and growing (turbid) should occur somewhere in each row, corresponding to a definite dilution of disinfectant.

The ratio:— 
$$\frac{\text{Sample (dilution for first clear tube)}}{\text{Phenol (dilution for first clear tube)}} = \text{phenol coefficient.}$$

The coefficient is shown by a table compiled from the dilution figures, reading down from the highest sample tube and across from the highest phenol tube that corresponds to the end-point in the broth subcultures. For example:

		Dilutions of sample—1 in:—								etc.
		589	650	729	833	946	1078	1200	1356	
		Give coefficients:—								
Dilutions of carbolic— 1 in:—	88·90	6·6	7·3	8·2	9·4	10·6	12·1	13·5	15·3	and so on.
	92·45	6·4	7·0	7·9	9·0	10·2	11·7	13·0	14·7	
	96·15	6·1	6·8	7·6	8·7	9·8	11·2	12·5	14·1	
	100	5·9	6·5	7·3	8·3	9·5	10·8	12·0	13·6	
	104	5·7	6·2	7·0	8·0	9·1	10·4	11·5	13·1	
	108·16	5·4	6·0	6·7	7·7	8·7	10·0	11·1	12·5	
	112·48	5·2	5·8	6·5	7·4	8·4	9·6	10·7	12·0	
	116·98	5·0	5·6	6·2	7·1	8·1	9·2	10·3	11·6	
	121·66	4·8	5·3	6·0	6·9	7·8	8·9	9·9	11·2	
	126·53	4·7	5·1	5·8	6·6	7·3	8·5	9·5	10·7	
etc.										

The fluids kill up to, and including, the furthest clear tube from the "killing end" of the rack.

For range of coefficient low to high, read one down and one across from dilution corresponding to clear tube.

Thus: If carbolic kills in 116·98 and does not kill in 121·66, and sample kills in 729 but not in 833, the reading lies between 6·0 and 7·1.

The limits of coefficient by this method are:

Upper limit = 
$$\frac{\text{lowest dilution where disinfectant failed to kill}}{\text{highest dilution where phenol killed}}$$

Lower limit = 
$$\frac{\text{highest dilution where disinfectant killed}}{\text{lowest dilution where phenol failed}}$$



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## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

## SOME NEW COLOUR REACTIONS WITH CERTAIN OILS AND FATS.

A NUMBER of aromatic hydroxy-compounds, *e.g.* phenol, resorcinol, menthol, phloroglucinol, salicylic acid, etc., give well-marked colour reactions with certain oils and fats, in the presence of a slight excess of bromine, in chloroformic solution. Resorcinol is a convenient reagent, being slightly soluble in chloroform, so that a saturated solution is easily prepared. The test is carried out as follows:

About 3 drops of the oil, or melted fat, are added to the resorcinol solution, followed by 5 drops of fairly strongly brominated hydrochloric acid. On shaking, a colour gradually forms with the reacting oil.

Cod-liver oil gives a pink coloration, which gradually turns to a deep red on standing. Of the wide range of oils and fats tested, butter-fat, whale oil, seal oil, oleo-oil, soya bean oil and rape oil give varying shades of pink. Linseed oil gives a deep green, and palm oil a light green coloration. Oils giving no reaction include olive oil, coconut oil and almond oil.

It seems probable that these phenolic reagents react with those oils which are said to contain vitamin A, and which also react with Rosenheim and Drummond's

antimony or arsenic trichloride reagent. Cod-liver oil gives by far the most pronounced coloration, butter fat coming next, though it is not nearly so reactive as cod-liver oil.

The insoluble fatty acids separated from cod-liver oil give no definite colour. The unsaponifiable and alcohol-soluble portions, however, after careful separation of free oil, give colour reactions with the phenolic reagents.

A. F. MCCARLEY.

BIRLING, HARTON MOOR LANE,  
SOUTH SHIELDS.

## INVESTIGATION OF THE OCCURRENCE OF *B. ABORTUS* (BANG) IN THE MILK OF ENGLISH HERDS (COUNTY OF SOMERSET).

THE milk (100 c.c.) from each herd was centrifuged and the deposit injected subcutaneously into a guinea-pig. After about four weeks the guinea-pig was killed and its spleen removed with precautions to ensure sterility. Cultivations were made from the interior of the spleen on glucose, neutral-red, vitamin agar in an atmosphere of about 10 per cent. of carbon dioxide and 90 per cent. of air at 37° C.

The glucose and neutral-red show at once whether any growth that may be obtained is due to contamination with *B. coli*. Usually no growth was obtained.

The number of herds so tested was 379 (confined to the County of Somerset), and *B. abortus* was demonstrated in the milk of 4. It was identified by its failure to grow, except at reduced oxygen tension, and by agglutination with standard anti-serum provided by the Medical Research Council. No further steps were taken to track down individual cows. These experiments were carried out concurrently with the examination of the milk for tubercle bacilli, on the suggestion of Dr. W. G. Savage.

COUNTY LABORATORY,  
WESTON-SUPER-MARE.

D. R. WOOD.  
E. T. ILLING.

## Official Appointment.

THE Minister of Health has confirmed the following appointment:

Mr. SYDNEY EMSLEY, B.Sc., F.I.C., as Public Analyst for the County of Isle of Wight (December 5, 1930).

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### LINIMENT OF TURPENTINE.

ON November 18, a Bethnal Green druggist was summoned at Old Street Police Court for having sold liniment of turpentine which was deficient in rectified oil of turpentine to the extent of 38 per cent.

Mr. Glyn Jones, for the defence, said that people who asked for liniment of turpentine often mean white liniment, which was not prepared in accordance with the British Pharmacopoeia.

The Public Analyst (Mr. A. E. Parkes), replying to the Magistrate (Mr. Clarke Hall), agreed that there was a considerable sale for preparations not so strong as the B.P. liniment.

The Magistrate said that, as many people wanted the white liniment, and not the B.P. preparation, he thought that the case would be met by payment of three guineas costs.

### SYRUP OF FIGS.

A TRADESMAN was summoned at Eglinton (Donegal) for selling syrup of figs which contained a foreign ingredient, namely, 0.01 per cent. of salicylic acid. The certificate of the Public Analyst was produced.

The solicitor for the defence laid stress upon the point that syrup of figs was not a food, and that, therefore, the prosecution must fail. The manufacturers described it as compound syrup of figs, which meant that it contained various ingredients. The salicylic acid was added as an antiseptic.

The Chairman of the Bench said that the Magistrates, having considered the legal definitions, were not prepared to hold that syrup of figs was a food, and the case would, therefore, be dismissed.

It was intimated that a further prosecution for selling an adulterated drug would probably be instituted.

## Ceylon.

### REPORT OF THE AGRICULTURAL ANALYST FOR THE YEAR 1929.

IN addition to advisory work on all matters pertaining to soils, manures and fertilisers, 361 samples from experiment stations or private persons were examined in the laboratories, which are under the direction of Mr. A. W. R. Joachim, B.Sc., A.I.C. Special investigations were also undertaken in connection with soil erosion, green manures, sugar cane products, coconut products, drugs, insecticides, waters, etc.

**CITRONELLA OIL INVESTIGATIONS.**—An investigation to determine the relationship between the chemical and physical characteristics of local citronella oils, the Schimmel's test on which Ceylon oils are generally sold, and market price showed that there was no connection between physical characteristics and market price, but that oils with the higher geraniol content fetched, on the whole, higher prices. Schimmel's test was found unreliable as a means of gauging the quality and purity of citronella oils, and the sale of those oils on the basis of the test alone is unsatisfactory. The purchase of the oil, on the basis of geraniol content, in addition to Schimmel's test, is recommended.

**RELATION OF SOIL REACTION TO VEGETATION.**—A preliminary study of the relation of soil reaction ( $pH$  value) to grass-land vegetation in Nalanda-Dambulla district was made in co-operation with the Systematic Botanist, and interesting results have already been obtained. Leguminous species and certain grasses occurred with greatest frequency on soils with  $pH$  about 7, while other grasses

occurred only in areas with  $pH$  of 6 and less. The greatest number and frequency of species occurred where the soil was about neutral, *i.e.* with  $pH$  7, and the least where the soil was most acidic in reaction. The  $pH$  determinations were made by means of the Bijlmann hydrogen electrode.

## Water Pollution Research Board.

### REPORT FOR THE YEAR ENDED 30TH JUNE, 1930, WITH REPORT OF THE DIRECTOR OF WATER POLLUTION RESEARCH.\*

A LARGE part of the Third Annual Report, which is on similar lines to that of last year (ANALYST, 1930, 55, 510), is concerned with the *Survey of the River Tees* as a typical river. The biological and chemical surveys of the tidal and non-tidal reaches have been extended for another year, but the hydrographical survey has been practically concluded, and a separate report is being prepared dealing with the measurements recorded. The flood appears to be at its greatest strength at a depth below one fathom, and the ebb is strongest on the surface. The volume of water moving down river in the top layer is greater than the volume moving up in the lower layers. Mixing of water in the different layers does occur, but any matter heavier than water, as it sinks, will tend to be carried up river. Biological and chemical work on the tidal reaches has indicated a stretch of polluted water containing less than the normal amount of dissolved oxygen, the centre of which is between Stockton and Newport at high water, and near the Transporter Bridge at Middlesbro' at low water. The small number of living forms in the central portion of the estuary and their complete absence in the bottom mud between Stockton and Newport may be due to this deoxygenated and polluted water, or to the fact that this is also the area of greatest variability in salinity. The decomposition of the organic matter of the sewage effluents discharged into the river is at its maximum in the hot summer, and this markedly affects the dissolved oxygen. The study of the algae and their distribution and seasonal variation is being continued, and the zoological work has shown the presence of about 70 different species.

**BEET SUGAR EFFLUENT.**—This work was continued, and it is concluded that percolating filters can be operated satisfactorily through a beet sugar campaign for the purification of pulp press liquor suitably diluted with river water or with effluent from the filters. A satisfactory rate of filtration (effecting an 80 to 90 per cent. purification) is about 100 gallons per cubic yard of filtering material per day for a pulp press liquor diluted to give a mixture with a five days' oxygen absorption value of 60 to 70 parts per 100,000. The laboratory experiments at Rothamsted have led to the conclusion that the partial conversion of sucrose into acid products assists the biological filtration, and the oxidation of the acid products is encouraged by the addition of nitrogen as albumin or as ammonium salt.

**BASE-EXCHANGE OR ZEOLITE PROCESS OF WATER SOFTENING.**—The investigation of the process has been continued and some results already published (Martin, *Chem. and Ind.*, 1930, 49, 389r). The influence on the process of variation in the temperature of the water, and in the concentration of dissolved calcium, magnesium, and sodium salts is now being investigated. The base exchange process makes no material difference in the bacterial population of the water.

\* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 9d. net.

**OTHER INVESTIGATIONS.**—An investigation into the treatment of *Corrosive and Plumbo-solvent waters* is being arranged, and the work now being done on *Colloids in connection with Sewage Disposal* is to include an examination of colloids in waste beet sugar factory waters.

**CO-ORDINATION OF RESEARCH.**—The Research Sub-Committee of the Institution of Gas Engineers has issued its 4th Report, which includes the various suggested methods for removal of phenols from liquor effluents from gas works, and an account of observations on the biological purification of sewage at Cheltenham containing definite quantities of spent gas liquor.

D. G. H.

## Ministry of Agriculture and Fisheries.

### STATUTORY RULES AND ORDERS, 1930, No. 370.\*

#### AGRICULTURAL PRODUCE (GRADING AND MARKING), ENGLAND.

THE AGRICULTURAL PRODUCE (GRADING AND MARKING) (CANNED FRUITS, PEAS AND BEANS) REGULATIONS, 1930, DATED JUNE 4, 1930, MADE BY THE MINISTER OF AGRICULTURE AND FISHERIES AS TO GRADE DESIGNATIONS AND GRADE DESIGNATION MARKS FOR CANNED FRUITS, CANNED PEAS AND CANNED BEANS.

In exercise of the powers conferred on him by the Agricultural Produce (Grading and Marking) Act, 1928, the Minister of Agriculture and Fisheries hereby makes the following regulations:—

1. Grade designations to indicate the quality of plums, strawberries, loganberries, raspberries, blackcurrants, gooseberries and apples produced and canned in England and Wales shall be as follows:—

SELECT DESSERT PLUMS  
SELECT STRAWBERRIES  
SELECT LOGANBERRIES  
SELECT RASPBERRIES  
SELECT BLACKCURRANTS  
SELECT DESSERT GOOSEBERRIES  
SELECT APPLES

and the quality indicated by such grade designations shall be deemed to be as described in column 2 of the First Schedule hereto.

2. A grade designation to indicate the quality of peas produced and canned in England and Wales shall be as follows:—

SELECT PEAS

and the quality indicated by such grade designation shall be deemed to be as described in column 2 of the Second Schedule hereto.

3. A grade designation to indicate the quality of beans produced and canned in England and Wales shall be as follows:—

SELECT BEANS

and the quality indicated by such grade designation shall be deemed to be as described in column 2 of the Third Schedule hereto.

4. A grade designation mark shall be one of the grade designations specified in regulations 1, 2 and 3 above, associated with the words "Empire Buying Begins at Home" and with the following mark, namely, a map of England and Wales, in silhouette, with the words, "Produce of England and Wales" inscribed in a circle placed centrally in the map within which circle is a design representing the Union Jack and which is more particularly described in the Fourth Schedule hereto.

5. These regulations shall come into operation on the 4th June, 1930.

6. These regulations may be cited as the Agricultural Produce (Grading and Marking) (Canned Fruits, Peas and Beans) Regulations, 1930.

In Witness whereof the Official Seal of the Minister of Agriculture and Fisheries is hereunto affixed this 4th day of June, 1930.

(L.S.)

CHARLES J. H. THOMAS.

SCHEDULE I.

FRUIT PRODUCED AND CANNED IN ENGLAND AND WALES: GRADE DESIGNATIONS AND DEFINITIONS OF QUALITY.

Grade Designation. 1.	Definition of Quality. 2.
Select Dessert Plums.	The fruit shall be firm, ripe, free from blemishes and reasonably uniform in size; it shall be canned in a syrup containing not less than 40 per cent. by weight of sugar (saccharine and glucose free) when packed; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.
Select Strawberries. Select Loganberries. Select Raspberries. Select Blackcurrants. Select Dessert Gooseberries.	The fruit shall be firm, ripe, free from blemishes and reasonably uniform in size; it shall be canned in a syrup containing not less than 45 per cent. by weight of sugar (saccharine and glucose free) when packed; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.
Select Apples.	The fruit shall be a recognised cooking variety, cored, peeled, and packed solid in slices of uniform size and colour; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.

SCHEDULE II.

PEAS PRODUCED AND CANNED IN ENGLAND AND WALES: GRADE DESIGNATION AND DEFINITION OF QUALITY.

Grade Designation. 1.	Definition of Quality. 2.
Select Peas.	The peas shall be undried, freshly gathered at the time of canning, of uniform variety, size and colour; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.

## SCHEDULE III.

## BEANS PRODUCED AND CANNED IN ENGLAND AND WALES: GRADE DESIGNATION AND DEFINITION OF QUALITY.

Grade Designation. 1.	Definition of Quality. 2.
Select Beans.	The beans shall be freshly gathered at the time of canning, of uniform variety, size and colour; no preservatives and/or artificial colouring agents shall be present other than those permitted under the Public Health (Preservatives, etc., in Food) Regulations in force for the time being.

## SCHEDULE IV.

## GRADE DESIGNATION MARK.

The Mark shall be a grade designation mark when used in association with a grade designation and with the words "Empire Buying Begins at Home."

## Ministry of Health.

### SALE OF FOOD AND DRUGS ACT.

#### EXTRACTS FROM THE ANNUAL REPORT FOR 1929—1930, AND ABSTRACT OF REPORTS OF PUBLIC ANALYSTS FOR THE YEAR 1929.\*

OF a total of 133,584 samples analysed (an increase of 4550 over 1928, *cf.* ANALYST, 1929, 55, 44), 7260, or 5·4 per cent., were reported against, and of these, 511 were contraventions of the Public Health Preservatives Regulations. Boric acid was present in foods, and sausages and other meat products were the main offending articles.

**MILK.**—Of 68,115 samples of milk, 5293 (7·8 per cent.) were reported against, a decrease over the previous year (8·2 per cent.). If "appeal to cow" samples are excluded, the percentage would be 7·5. One milk was 43·3 per cent. deficient in fat, and a fine of £50 was imposed; in another instance a fine of £25 was imposed for a deficiency of 10·3 per cent. Annatto was present in 10 samples, 22 samples contained dirt, and formaldehyde or boric acid was present in 5 cases. Added water was reported in 13 cases in separated or skimmed milk. Of 226 samples of dried milk, only 3 were reported against, and 41 of 1294 samples of condensed milk were defective, mostly in fat.

**CREAM.**—Thirty-seven of 51 adulterated creams, from a total of 2368 samples examined, contained boron preservative. Two "fresh" creams were artificial or reconstituted; 10 tinned creams were deficient in milk fat or solids, and 95 per cent. of the fat of a restaurant cream was not derived from milk.

**BUTTER AND MARGARINE.**—Only 149 of 10,965 samples of butter were reported as adulterated, and, of these, 81 contained excess water, 47 consisted wholly or in part of foreign fats, 19 contained boron preservative, 1 cane sugar, and 1 traces

\* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 3d. net.

of sand. Of 60 of 3951 margarines, 21 contained excess water, 3 boric acid, 1 had been attacked by fungoid growth, and one contained 85 per cent. of butter fat.

**LARD.**—Seven of 3152 lards, 20 of 553 drippings, and 12 of 212 suets were adulterated. Two lards contained free fatty acids, and 5 were not derived wholly from pig's fat; the drippings mostly contained excess of water and free fatty acids, but one consisted wholly of vegetable fat, and excess of rice flour was present in suets.

**CHEESE.**—Of 1321 cheeses, 27 were adulterated. Cream cheese was ordinary whole-milk cheese; skimmed milk cheese was sold as Cheshire; 2 Gorgonzolas had a covering, equivalent to 10 per cent. of the cheese, consisting of 21 per cent. of tallow and 79 per cent. of barytes coloured with oxide of iron. No tin-wrapped cheese examined was free from contamination by tin.

**BREAD AND FLOUR.**—Three samples of flour (of 543) and 14 of self-raising flour (of 723) were reported against; 2 flours contained arsenious oxide and 1 ammonium sulphate, and one sample of self-raising and one of "diabetic" flour were ordinary flours.

**OTHER FOODS.**—Thirty-one of 1324 samples of *jams and marmalade* were reported against, 21 for containing excess sulphur dioxide, 2 for glucose, 6 for apple pulp or fruit juices other than indicated on the label, and one sample was decomposed. The sale of artificial for malt vinegar and deficiency in acetic acid accounted for 142 of 1856 *vinegar samples* examined. Of 2444 samples of *spirits*, 275 were reported against as more than 35 degrees under proof. Fifteen of 548 beers contained lead or excess of sulphur dioxide. In 2 cases, 199 and 366 parts respectively, of sulphur dioxide per million were present. Other articles of food examined included 1131 *cocoas* (3 contained extraneous mineral matter); 1692 *coffees* (8 contained chicory); 961 *sugars*; 2940 samples of *sausages, polonies*, etc., of which 265 contained sulphur dioxide not indicated on the label; 10 were deficient in meat and some contained boric acid. One sample of *non-alcoholic wine* consisted of sugar and water acidified and coloured with an aniline dye. Copper sulphate was present in 9 samples of food, and tin in 19 of tinned fruits, fish, etc.

**DRUGS.**—The 5544 samples of drugs represented 109 different kinds, and 254 were reported as adulterated. These included several camphorated oils deficient in camphor, and one made from mineral instead of olive oil; potassium iodide; distilled water which was ordinary water; cod-liver oil tablets devoid of vitamin A; 31 samples of sweet spirit of nitre deficient in ethyl nitrite, and short weight packets of 13 Seidlitz powders.

D. G. H.

## Department of Scientific and Industrial Research.

### FUEL RESEARCH. Technical Paper, No. 27.

#### MEASUREMENT OF A RAPIDLY FLUCTUATING FLOW OF GAS.\*

THE apparatus described measures and records the volume of gas made during each part or cycle of the normal water-gas process, or during the whole operation,

\* By J. G. King and B. H. Williams. Obtainable at His Majesty's Stationery Office, Adastral House, Kingsway, London, W.C.2. Price 6d. net.



and in conjunction with an orifice-plate is recommended for recording variable gas-flow in industrial processes. It is to be used for future investigations of this nature at the Fuel Research Station.

In the present instance the average rate of gas-flow was about 300 cb. ft. per minute, and the process was subject to periodic interruptions for the purpose of removing clinker from the generator. The period of the test was further divided into 14 periods, at the beginning of which coke was charged into the generator, and finally, sub-divided into 6 cycles comprising 1 minute's blow and 4 minutes' gas-making.

**GAS-FLOW.**—Preliminary experiments were made with an orifice-plate and Pitot tube. The latter was not accurate enough for the small differences and rapid variations in pressure involved, and the former was used, employing Hodgson's formula, the simplified form of which was in the present instance  $Q=170\sqrt{P/d}$ , where  $Q$  is the gas-flow in cb. ft. per minute at 760 mm. and 15° C.,  $P$  the pressure-drop across the orifice in inches of water, and  $d$  the density of the gas at the orifice in lbs. per cb. ft.

$P$  was found as a function of time by means of a sensitive inclined gauge tube, 2.5 mm. wide, containing commercial aniline, and giving a movement of 4 inches for a pressure-change equivalent to 0.5 inch of water. A photographic record was obtained by arranging that the meniscus should move along the tube between a beam of light, and a slit in a light-tight box, behind which was fixed a perforated copper strip and sensitive photographic paper moving round a roller in a direction at right angles to the slit. The perforations, which were 0.3 mm. in diameter and 1 mm. apart, produced a series of parallel straight lines on the chart, which were interrupted by a movement of the opaque gauge-liquid between the paper and the light. The range of the instrument was altered by changing the angle of slope of the tube, and from the speed of movement of the paper and the relation between the pressure-difference at the orifice plate and the movement of the gauge,  $Q$  could be calculated. Typical diagrams are shown and the corresponding calculations given.

Calibrations of the gauge against known static pressures showed that the motion of the liquid was proportional to the static pressure for 0 to 0.5 inch of water, errors due to change in shape of meniscus and shrinkage of the photographic paper being negligible. Comparison with values obtained by means of a calibrated gas-holder showed differences varying from +1.77 to -1.89 per cent. for 10 individual cycles, the total difference for the whole experiment being -0.13 per cent.

**AIR-FLOW.**—It has hitherto been difficult to measure the flow of air in such experiments, owing to the large and fluctuating volumes concerned, so that the volume used during the "blow" period of the water-gas process is usually obtained by calculation from the carbon balance, and is thus based on a difference figure. The extent of the error involved is 5 to 10 per cent. of the thermal value of the coke with which the generator is charged.

Orifice plate and Pitot tube methods applied to exact measurements of air supplied during the blow period are, therefore, described. The agreement is of the order of 1 per cent., but it is concluded that the former method, though not necessarily more accurate, is more practicable in such circumstances. Thus, the Pitot tube must be fixed in one position in the air-main, and a relation found to connect the readings with the pressure distribution along the remainder of the main. The orifice-plate responds rapidly to changes in rate of flow, and the pressure-differences across it are much bigger than those set up in the Pitot tube.

J. G.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

## Food and Drugs Analysis.

**Detection of Heated Milk.** K. Eble and H. Pfeiffer. (*Z. Unters. Lebensm.*, 1930, 60, 311-314.)—Variations of the benzidine test are described which, it is claimed, give less ambiguous results than the original method. Five c.c. of milk are shaken with 0.5 c.c. of a 4 per cent. solution of benzidine in 96 per cent. alcohol, 1 drop of 1 per cent. (by weight) hydrogen peroxide added after 1 minute, and the mixture again shaken. Raw milk gives a stable blue-green colour, slightly heated milk (e.g. 30 minutes at 63° C.) a similar but fading colour, whilst strongly-heated milk is colourless. After 1 minute, 10 c.c. of a saturated solution of magnesium sulphate are added, and the mixture shaken, when raw milk becomes grey-green and turns bluish-green in 10 minutes, whilst heated milk is grey-green and turns to greyish-blue. After a further 10 minutes, dark blue curds separate from slightly-heated milk, whilst in strongly heated milk the curds are white. Alternatively, 5 c.c. of milk, 10 c.c. of magnesium sulphate solution and 1 drop of peroxide may be mixed, and the benzidine added after 1 minute, when raw milk appears bright blue and milk heated at 63° C. bright green, both colours becoming deeper on standing. If magnesium sulphate is replaced by sodium sulphate, the respective colours are chocolate to violet-brown and green-brown. Heated milk having an abnormally high leucocyte content or lactic acid acidity, or heated for a short period below 63° C., tends to give the colour reactions of raw milks, and it is advisable before making the test to add 0.25 *N* potassium hydroxide solution till the acidity is 6°.\* These exceptions are due to the peroxidase associated with the proteins or leucocytes of the milk which normally passes into solution when the milk is heated, and so loses its activity, but may be re-activated by lactic acid, etc., and inactivated by addition of amyl alcohol. In doubtful cases a mixture of 10 c.c. of milk, 10 c.c. of a saturated solution of magnesium sulphate and 1 c.c. of 20 per cent. acetic acid is shaken and centrifuged for 6 minutes at 120 revs. per minute. Slightly-heated milk gives a clear serum, raw milk a definitely opalescent serum, and mixed milk containing 20 per cent. or more of raw milk a faint turbidity. J. G.

**Phosphatide Contents of Varieties of Bread.** B. Rewald. (*Z. Unters. Lebensm.*, 1930, 60, 315-318.)—The phosphatide content was determined by the author's method (*Biochem. Z.*, 1928, 198, 103; 202, 394), the sample being extracted in succession with a large excess of acetone and a mixture of benzene and alcohol. The extracts were evaporated separately, the residues extracted with dry ether, and the phosphorus determined in each case in the residue after evaporation of the ether. The sum of these two figures was taken as the total phosphatide content, the following values (percentages on the dry sample) being found:—Rye bread-crumbs 0.174 (crust 0.174), white bread (crust+crumb) 0.287, pumpnickel

\* One degree = 0.01 per cent. of acidity as lactic acid.

(crust+crumb) 0.073, black bread-crumb 0.183 (crust 0.194), Graham bread-crumb 0.267 (crust 0.302). The respective phosphorus contents of the residues after extraction were 0.20, 0.19, 0.24, 0.32, 0.34, 0.35, 0.30, and 0.29 per cent. With the exception of pumpernickel, therefore, the different breads examined contain approximately the same amounts of phosphatide, whilst the phosphorus contents of the residues are approximately equal in all cases. The fact that almost equal quantities are found in crust and crumb indicates that temperature is without influence on the phosphatide content, and thus confirms the conclusions resulting from a study of phosphatides of hard-boiled eggs, though, in the case of bread, the phosphatide isolated is a typical plant product. J. G.

**Effect of Sodium Bisulphite on the Polarising Power of Sugars.** Y. Tomoda and T. Taguchi. (*J. Soc. Chem. Ind. Japan*, 1930, 33, 434B.)—A table is given showing the effect of sodium bisulphite on eleven sugars, the polarising powers of five of which are markedly affected (*d*-glucose, *l*-xylose, *l*-arabinose, *d*-galactose, and lactose), whilst the polarising powers of the other six are hardly affected at all (maltose, *d*-mannose, sucrose, raffinose, dextrin, *d*-fructose). For instance, the polarising power of glucose is decreased from +15.4° V. to -0.3° V., but that of fructose only from -26.8 to -26.5. This property of sodium bisulphite can be utilised in the analysis of mixtures of sugars containing glucose. Thirty grms. of sodium bisulphite are effective for 100 c.c. of the sugar solution containing 2.5 to 24 per cent. of glucose. If a saccharimeter reading be taken with a 200 mm. tube of a mixed solution of glucose and fructose, in the absence and in the presence of sodium bisulphite, the amounts of glucose and fructose can be calculated from the formula:

$$x = 0.3225 \times (P - P')$$

$$y = 0.1859 \times (-P')$$

where  $x$  = grms. of glucose per 100 c.c.

$y$  = grms. of fructose per 100 c.c.

$P$  = saccharimeter reading °V. at 20° C. in 200 mm. tube

$P'$  = ditto in the presence of 30 grms. of sodium bisulphite.

The composition of invert sugar can be found in a similar manner after neutralisation with sodium bicarbonate.

In the case of mixtures of glucose and maltose a modified formulae has to be used, since the polarising power of maltose is decreased to a somewhat greater extent (+37.2° V. to +30.9° V.) than that of fructose. Where  $x$  and  $y$  represent the grms. per 100 c.c. of glucose and maltose, respectively—

$$x = 0.3225 \times \left( P - \frac{p}{p'} P' \right)$$

$$y = 0.1247 \times \left( \frac{p}{p'} P' \right)$$

where  $P$  and  $P'$  represent saccharimeter readings of the mixed solution in the absence and in the presence of sodium bisulphite;  $p$  and  $p'$ , saccharimeter readings

of a maltose solution at a certain concentration in the presence and absence respectively of sodium bisulphite.

The method may also be applied to the analysis of honey and hydrolysed products of starch, etc. R. F. I.

**Detection of Isopropyl Alcohol in Brandy, Spirits, Tinctures, Cosmetics and Liniments by means of Piperonal.** G. Reif. (*Z. Unters. Lebensm.*, 1930, 191, 243-254.)—The author's method (*id.*, 1929, 57, 277; *ANALYST*, 1928, 53, 497) has been tested with 124 prepared or purchased liniments (the compositions and uses of which are described), 25 samples of brandy, 40 of cosmetics, and a number of spirits and tinctures representing many different types, and the following general procedure found satisfactory:—The sample (10 c.c.) is distilled on the water-bath into a small cylinder immersed in ice-water till no more alcohol is evolved, 0.3 c.c. of distillate shaken with 0.7 c.c. of a mixture of 80 c.c. of absolute alcohol and 20 c.c. of water, and a solution of 0.1 gm. of hydroxylamine hydrochloride in 3 c.c. of water added. The mixture is shaken for 3 minutes at the ordinary temperature, 0.4 gm. of absorbent charcoal (*carbo medicinalis*) added, the mixture shaken and filtered through a small dry paper into a 100 c.c. round-bottomed flask. Five c.c. of a freshly-prepared solution of 0.5 gm. of piperonal in 100 c.c. of absolute alcohol are added, followed by the slow addition, to prevent boiling, of 20 c.c. of sulphuric acid (sp. gr. 1.84). The mixture is shaken, placed on the water-bath for 5 minutes in a large test-tube, when a red colour indicates the presence of *iso*-propyl alcohol, and a green-brown colour its absence. If 30 c.c. of 30 per cent. acetic acid are then added, a rose to red colour, stable for 30 minutes, is a positive reaction (brown in the presence of small amounts of the alcohol), whilst a colourless solution or weak red tinge stable for a minute and turning yellow-grey or colourless shows the alcohol to be absent. The sensitiveness is, in general, 1 to 2 per cent., but 0.1 per cent. for liniments. With these formaldehyde, if present, must be destroyed before distillation by heating the sample with 5 c.c. of sodium hydroxide solution (sp. gr. 1.125) under a reflux condenser for 1 hour. Less than 20 per cent. of acetone has no influence on the reaction.

J. G.

**Composition and Determination of Water in Tomatoes and Preserved Tomatoes.** A. Leonhard. (*Z. Unters. Lebensm.*, 1930, 60, 185-195.)—The various methods of determination of the dry solids in tomato-products, and their disadvantages, are discussed. The author prefers to obtain the percentage by difference from the water-content determined on 5 to 10 grms. of the mixed sample weighed in an aluminium boat and distilled with 150 c.c. of xylene in a 300 c.c. Erlenmeyer flask on a sand-bath. A delivery-tube, bent at 45° and 350 mm. long, leads into a vertical 150 c.c. graduated receiver, completely immersed in running water, and closed except for a glass tube drawn-out at one end to 2 mm. in diameter and inserted through a rubber stopper. The bottom of the receiver should contain 1 c.c. of 1:1 potassium hydroxide solution, and 150 c.c. should distil over in about

20 minutes. Any condensate is then removed from the delivery tube by application of a small flame, the contents of the receiver shaken gently, and the volume of the separated aqueous layer read after 2 hours, the 1 c.c. of alkali originally present being deducted. Any volatile oils are retained by the xylene. The results were compared for 40 samples of Italian and German origin with those calculated from the sp. gr. of the extract obtained by filtering a warm mixture of 20 grms. of sample and 100 c.c. of water, and washing the residue with small quantities of hot water up to a total filtrate of 200 c.c. This solution was also used for determinations of sugar, acidity, soluble acids, etc., the full analytical results being tabulated. In general, the dry solids obtained by the above method were lower than those obtained from the sp. gr., especially for samples of the more liquid type. The following table gives the percentage compositions of some typical tomato-products:—

Description.	Artificial colour.	Water (direct).	Dry residue (calc.).	Sp. gr. (1:10, filtered).	Dry residue (calc.) from sp. gr.).	Invert sugar.	Sucrose.	Acidity (c.c. N KOH).	Ash.	Alkalinity of ash (c.c. N KOH).	Chlorine.
Thin liquid purée (Swiss)	+	89.42	10.58	1.0082	10.6	3.72	0.65	11.0	2.39	—	0.87
Thin liquid sauce (German)	+	84.10	15.90	1.0070	18.2	7.64	0.79	11.0	2.60	—	0.90
Thin liquid purée (Swiss)	0	85.45	14.55	1.0133	17.5	6.57	1.62	9.2	3.33	10.5	1.56
Pulp (German)	0	83.85	16.15	1.0069	17.8	7.63	0	13.5	3.20	16.5	1.14
Solid pulp (Italian)	0	67.39	32.61	—	—	13.90	0	31.8	8.43	33.0	3.35
Extract (Italian)	—	64.10	35.90	1.0153	39.5	17.58	1.47	31.0	6.59	34.5	2.20
Almost solid purée (Italian)	0	74.38	25.62	1.0105	27.1	15.94	0.87	22.5	2.48	23.2	0.20
Solid pulp (Italian)	+(?)	71.55	28.45	1.0110	28.9	11.75	0	18.5	4.89	32.0	1.27
Solid pulp (Italian)	+	67.50	32.50	1.0140	36.2	13.66	0	32.0	7.40	33.2	2.78
Solid control-sample (German)	—	74.88	25.12	1.0104	26.2	12.98	0	21.0	3.74	—	1.16
Fresh pulp from 1929 crop (German)	—	92.4	7.6	1.0051	6.6	3.90	0	6.5	0.63	7.0	0.04
Do.	—	94.0	6.0	1.0040	5.2	2.56	0	5.2	0.50	5.5	0.04

J. G.

**Determination of Formic Acid in Fruit Juices.** A. Hanak and K. Kürschner. (*Z. Unters. Lebensm.*, 1930, **60**, 278–290.)—Ten c.c. of juice (or 10 grms. of fruit syrup diluted to 20 c.c.) are steam-distilled with 0.3 gm. of tartaric acid, so that after 45 minutes there is very little increase in the volume of liquid in the distillation flask, which should on no account exceed 20 c.c. Overheating is avoided by means of an asbestos shield, and 400 c.c. of distillate are collected in sufficient distilled water just to cover the end of the condenser adapter. The distillate is diluted to 500 c.c., 200 c.c. of which are titrated, and the total free, volatile acids calculated. The calculated amount of 0.5 *N* sodium hydroxide solution required to neutralise the acidity is then added to 100 or 200 c.c. of distillate (according to the amount of formic acid present), followed by 0.5 to 1 c.c. in excess. The mixture is warmed for 10 minutes to saponify any formic esters, and then evaporated with 0.2 gm. of sodium carbonate, without boiling, till 30 c.c. remain. A 0.2 *N* solution of potassium permanganate is added, so that

there is an excess of about 1 to 1.5 c.c. (3 and 5 c.c. being usually required for 100 and 200 c.c. of original solution, respectively). Oxidation is complete after 45 to 60 minutes, when 1 c.c. of 10 per cent. zinc sulphate solution is added to flocculate any suspended matter, and the solution diluted to 50 c.c. and filtered and the excess of permanganate determined colorimetrically. For this purpose the colour of 25 c.c. of filtrate is matched in a colorimeter against that of a 0.2 N solution of potassium permanganate. Since the sensitiveness of this colorimetric method is 0.1 c.c., and 1 c.c. of 0.2 N permanganate solution  $\equiv$  2.76 mgrms. of formic acid, the method should be accurate within  $\pm 0.276$  mgrms. Solutions containing 0.13 to 0.34 grms. of formic acid per 100 c.c. gave results with an error of 0 to +6 mgrms. Fincke's mercuric chloride method (*id.*, 1911, 21, 1; Reisser, *id.*, 1916, 96, 355), which is discussed critically, gave results 10 to 30 mgrms. low. The method was tested on apple juice (16.7 mgrms. per 100 c.c.), currant (40.45), bilberry (45.5), and other fruit juices. J. G.

**Koryo (Millet Seed) Oil.** S. Ueno and N. Kuzel. (*J. Soc. Chem. Ind. Japan*, 1930, 33, 452b.)—The oil used was extracted with ether from the ground grains of millet, *Andropogon sorghum vulgaris*, Hack (Manchu). It amounted to about 3 per cent. of the grains, was a dark brown paste having an odour resembling ethyl alcohol. The constants of the refined oil were:—Sp. gr. at 20/4° C., 0.9206;  $n_D^{20}$ , 1.4659; acid value, 40.0; saponification value, 183.0; unsaponifiable matter, 5.43 per cent.; m.pt. of unsaponifiable matter, 67.8 to 80.7° C.; with iodine value, 68.4; iodine value of the oil, 114.0. The lead salt and ether method showed the fatty acids to consist of 71.6 per cent. of liquid acids (iodine value 120), and 28.4 per cent. of solid acids (iodine value 13.1). The unsaponifiable matter appeared to contain considerable amounts of hydrocarbons (m.pt., 60.5 to 61.8°), higher alcohols and sterols. The unsaturated acids consisted of oleic and linolic in about the proportion of 2:1. The saturated acids were mostly palmitic with traces of myristic and stearic acids. R. F. I.

**Oil from the Seeds of *Asteriastigma Macrocarpa*.** D. H. Peacock and Chit Thoung. (*J. Soc. Chem. Ind.*, 1931, 50, 7–8t.)—The seeds of *Asteriastigma macrocarpa* are known in Burma as Kalaw seeds, a term which includes seeds of other species, most of which contain optically active fatty acids. (*Burma Forest Bull.*, No. 21.) The oil extracted by means of benzene, and dried, had: Sp. gr. 35° C., 0.9501;  $n_D^{20}$ , 1.4790°;  $[\alpha]_D^{20} + 52.8$ ; iodine value, 112.3; saponification value, 195.6; and unsaponifiable matter, 3 per cent. The oil was saponified with alcoholic potash, the potassium salt extracted with ether, the recovered acids dissolved in alcohol, and treated with half the requisite quantity of lead acetate. The precipitated salt contained no ether-soluble fraction. The alcoholic solution of the rest of the fatty acids was treated with excess of lead acetate, and the acid recovered from the ether treatment of the precipitated salts had a negligible rotatory power and an iodine value of 90, and was proved to be oleic acid (5.7 per cent. on the original oil). The mixture of fatty acids was converted to the acid chlorides and then the amides. The acids were esterified, and four fractions

were obtained distilling (1) below 210°; (2) 210°–220°; (3) 220°–225°; (4) 225°–235° C. After redistillation the rotations were (1)+37°; (2)+37°; (3)+41°; and (4)+44°. These fractions were redistilled twice; and eventually, after further analyses, it is concluded that a saturated acid, possibly palmitic, is present, and also chaulmoogric acid, or possibly a mixture of chaulmoogric and hydnocarpic (although the latter could not be isolated) to the extent of about 75 per cent. of the original fatty acids.

D. G. H.

**Quantitative Determination of Cholesterol and Lecithin. Evaluation of Egg Products.** J. Tillmans, H. Riffart and A. Kühn. (*Z. Unters. Lebensm.*, 1930, 60, 361–389.)—The methods suggested by other workers for these determinations are critically discussed, and the following procedure (after Szent-Györgyi) recommended for *cholesterol*:—A solution of not more than 4 mgrms. of cholesterol in 2 c.c. of warm acetone is evaporated on the water-bath with 1 c.c. of a 2 per cent. solution of pure digitonin in 80 per cent. alcohol till 1.5 c.c. remains. After 15 minutes at room-temperature the mixture is filtered on a sintered-glass crucible enclosed in a jacket through which steam can be passed, the precipitate transferred with acetone and given 2 washes with ether, followed by 3 with warm chloroform, 2 with ether, 1 with acetone, and 1 with cold water, 1.5 c.c. at a time. The suction is adjusted to filter 1 drop per second, and the precipitate should always be covered with liquid to protect it from the air. Steam is then passed through the jacket, 10 (1.5 c.c.) portions of water added, and the washing finished, using the full suction of the pump. The suction and heating are continued, 10 c.c. of a clear 1 per cent. solution of potassium dichromate in concentrated sulphuric acid pipetted slowly on to the precipitate so as to fill one-fourth of the crucible, the crucible washed with 3 (1 c.c.) portions of cold water, sucked well and the total filtrate collected. After 1 hour oxidation is complete; 100 c.c. of water and 10 c.c. of 5 per cent. potassium iodide solution are added, and the liberated iodine titrated with 0.1 *N* sodium thiosulphate solution, using 10 drops of 1 per cent. starch solution for every 10 c.c. of chromic acid taken, allowance being made for the blank titration. The mean results of 15 experiments with pure cholesterol showed that the volume of thiosulphate solution equivalent to the chromic acid used up, divided by 8.7, gives the cholesterol content (in mgrms.) for 0.5 to 4 mgrms. The factor 7.9 was found for phytosterol. The m.p.s. were 145° and 136° C. for the two sterols, recrystallised from absolute alcohol, and 114° and 126° C. for their acetates, respectively.

Egg-products are first dried on the water-bath with sand, extracted in a Soxhlet apparatus with ether, and the residue, after evaporation, dried at 100° C. and weighed. A weighed portion is then dissolved in warm acetone, the solution filtered, diluted to a suitable volume with acetone, and the above procedure followed. This gives "free" cholesterol. Another portion of the ethereal extract is then saponified on the water-bath for 1 hour with a 20 per cent. alcoholic solution of potassium hydroxide (or by heating with 20 c.c. of 5 per cent. sodium alcoholate for 4 hours), the residue dried, mixed with sand and extracted as before, to give the

cholesterol after saponification. The results obtained for 8 samples of egg-products are slightly higher than those of other workers, and a mean value of 239 mgrms. of cholesterol, calculated on the basis of an egg-yolk weighing 16 grms., was found (without saponification). After saponification the values were 10 to 80 mgrms. higher. Pastry is ground, 20 grms. extracted for 6 hours with ether, and the above method applied. Various products gave 10.2 to 25.6 mgrms. per 100 grms. of dry sample, whilst vermicelli, etc., containing dried or fresh egg contained 81 to 140 mgrms. per 100 grms. of dry material. Fats are melted and filtered, and 0.2 gm. dissolved in 2 c.c. of acetone. In such cases the digitonin precipitate should be washed with ether (5 times), warm chloroform (5), ether (3) and twice with acetone (1.5 c.c. each time). The results agree well with those of Bömer (*id.*, 1901, 4, 1070), but are much higher than those of other workers, *viz.* 140 to 370 mgrms. per 100 grms. for butter, lard, beef-fat, etc.

*Lecithin* should be determined as follows (*cf.* Juckenack, *id.*, 1904, 8, 94):—The powdered sample (10 grms.) is extracted for 3 hours with hot alcohol in a Soxhlet apparatus, and the residue left on evaporation of the extract is warmed with 15 c.c. of perhydrol and 5 to 10 c.c. of concentrated sulphuric acid till brown in colour. The cool mixture is diluted to 100 c.c., 25 c.c. neutralised with ammonia to methyl orange, and the lecithin phosphate precipitated in the cold in a volume of 60 c.c. with 5 c.c. of 1.5 per cent. strychnine nitrate solution and 15 c.c. of a mixture of 33.33 grms. of ammonium molybdate in 100 c.c. of water and 300 c.c. of dilute nitric acid containing 200 c.c. of acid of sp. gr. 1.4 (*cf.* Embden, *Z. physiol. Chem.*, 1921, 113, 138). After 20 minutes the precipitate is filtered off on a weighed crucible, washed with 25 c.c. of the ice-cold mixed reagents (diluted 5-fold), and then with ice-water till free from acid. The weight of precipitate (dried at 100° C.) divided by 39 gives  $P_2O_5$ , and the method has an accuracy of 0.02 mgrm. for 0.5 to 4 mgrms. of  $P_2O_5$ . The agreement with Juckenack's method (*loc cit.*) is good, but the colorimetric method of Misson (*Chem. Ztg.*, 1908, 32, 633) gives higher results. The contents of water, cholesterol and lecithin (found by the above method and by Juckenack's method, *loc cit.*), are tabulated for 21 types of pastry-products containing eggs, together with tests for artificial colouring matters. A table is also given for the calculation of the egg content of a sample from the cholesterol content, 58.7 and 71.4 mgrms. per 100 grms. of ordinary and hard (e.g. macaroni) pastry, respectively, corresponding with 1 egg per 500 grms. of flour, and 151.5 and 164.2 mgrms. respectively, with 3 eggs. Storage of such trade-samples for 1 year produced no reduction in cholesterol or lecithin content, but home-made products showed a marked decrease, especially in lecithin content. This fall is attributed to the influence of exposure to light and to the water content. Tollens' reagent (*Z. Unters. Lebensm.*, 1904, 7, 22) and Popp's ultra-violet light method (*ANALYST*, 1926, 51, 540) were found unreliable for differentiating the various types of protein in pastry. In general, "free" lecithin, determined on an extract of the sample in petroleum spirit, was found to be less for preserved than for fresh samples, but the method is not reliable for the purposes of differentiation.

J. G.



**Determination of Nitrite and Sulphite in the Presence of one another in Salt Mixtures and in Meat Products.** E. Szabó. (*Z. Unters. Lebensm.*, 1930, 60, 389-395.)—The salt is dissolved in a little boiled water, sodium carbonate added, and the sulphite precipitated by a cold solution of lead acetate or nitrate. The mixture is diluted to a known volume, filtered, and an aliquot portion taken for the colorimetric determination of the nitrite by means of *m*-phenylene-diamine. The precipitate is washed with cold water, suspended in cold boiled water, and the sulphur dioxide determined by acidification and distillation in a stream of carbon dioxide in the usual way. Alternatively, the sample may be dissolved in a dilute solution of sodium bicarbonate, and the sulphur dioxide determined by addition of a slight excess of 0.1 *N* iodine solution and back-titration with 0.1 *N* sodium thiosulphate solution ( $\text{Na}_2\text{SO}_3 + \text{I}_2 + \text{H}_2\text{O} = \text{Na}_2\text{SO}_4 + 2\text{HI}$ ). The air is then removed by means of a stream of carbon dioxide, the solution acidified with 1 c.c. of 0.1 *N* sulphuric acid, and after 1 minute (during which period the beaker of liquid should not be disturbed, so as to retain the carbon dioxide and exclude air) the free iodine is titrated with the thiosulphate solution ( $2\text{HNO}_3 + 2\text{HI} = 2\text{H}_2\text{O} + 2\text{NO} + \text{I}_2$ ). The stream of carbon dioxide serves to mix the solutions. Potassium iodide solution should be added in the second titration only if the amount of nitrite is in great excess. A maximum error of approximately  $\pm 1$  mgrm. is recorded for both determinations in the case of solutions containing mixtures of 65 to 100 mgrms. of sodium sulphite and 25 to 100 mgrms. of sodium nitrite. Meat samples are extracted with sodium bicarbonate solution, and the extract should be free from substances which may interfere with the iodimetric titration. J. G.

**Potentiometric Determination of Alkaloids by Means of Potassium Iodomercurate.** L. Maricq. (*Bull. Soc. Chim. Belg.*, 1930, 39, 496-502.)—An extension of the author's method (*ANALYST*, 1930, 55, 284) is described in which the alkaloid is precipitated by means of potassium iodomercurate, the excess of which is determined potentiometrically by means of a standard solution of mercuric chloride. Four possible alkaloid iodomercurates of the type  $(\text{HgI}_2)_n\text{A}\cdot\text{HI}$  may be formed according to the number of molecules of mercuric iodide in the complex, and it is necessary to calculate an empirical coefficient for each alkaloid. Atropine is used as a solution of 0.01 to 0.02 grm. in 5 to 15 c.c. of 0.02 *N* sulphuric acid, and it is found that the factor 0.001446 gives results having a maximum relative error of 2 per cent. For hyoscyamine the same factor is used, and the maximum error is 16 per cent. For pilocarpine the factor is 0.00104 (error 1.1 per cent.), and there is evidence that, if the amount taken exceeds 0.01 grm., there is a high negative error which may, however, be eliminated by addition of a little solid mercuric iodide at the moment of precipitation, followed by vigorous shaking. This error, which occurs also with excessive quantities of atropine or hyoscyamine and may be eliminated by a similar procedure, is due to an insufficiency of mercuric iodide in the reagent used, resulting in a complex of the wrong composition which is redissolved by the hydriodic acid liberated by the reaction itself. For cinchonine and sparteine 5 to 15 c.c. of a solution of 0.005 to 0.015 grm. of alkaloid in 0.05

and 0.02 *N* sulphuric acid, respectively, are used, the factors being 0.00073 and 0.00058, and the errors 2.0 and 1.8 per cent., respectively. Since 1 c.c. of 0.005 *N* mercuric chloride solution corresponds with  $5 \times 10^{-6}$  grm. mol. of a monobasic alkaloid, the factors are given in each case by the product, molecular weight  $\times 5 \times 10^{-6}$ . The method is confirmed by the results which, in each case, correspond with the type of complex shown by other workers to be formed by the alkaloid concerned.

J. G.

**Identification of Alkaloids by Precipitation.** C. C. Fulton. (*J. Assoc. Off. Agric. Chem.*, 1930, 13, 491-497.)—A natural classification of the alkaloids, based solely on their precipitation from dilute solution, is described, the key to the classification being the relative sensitivity of different reagents to each alkaloid. Amines and similar basic substances precipitated by the same reagents are included in the scheme and, for convenience, the other reagents are compared with phosphomolybdic acid. The procedure employed is as follows: A drop of a solution either of the alkaloid salt in water or of the alkaloid itself in dilute acid (sulphuric) is treated on a microscope slide with a drop of 10 per cent. phosphomolybdic acid solution. If a heavy precipitate is formed, the alkaloid solution is diluted until no more than a distinct precipitate is obtained with the reagent. This standard solution, which is nearly always considerably weaker than 0.1 per cent., is tested similarly with other reagents.

Tests thus made on a number of alkaloids show that the reagents may be so arranged that the effect of many reagents can be predicted with certainty from the result obtained with the first one tried. For instance, if picric acid precipitates the alkaloid in the standard solution, then Mayer's reagent, gold chloride, and bromine water will also precipitate it; whilst if picric acid does not precipitate it, platinum chloride, potassium chromate, and potassium hydroxide will also fail. The alkaloids may be similarly arranged. In the following arrangement of some of the principal alkaloids and reagents, each alkaloid is precipitated in the standard solution by all the reagents preceding it and by none of those succeeding it: phosphomolybdic acid, caffeine, Wagner's reagent, atropine, gold chloride, morphine, Marme's reagent, cocaine, picric acid, diacetylmorphine (heroine), ammonium molybdate, quinine, platinum chloride, strychnine, potassium chromate, papaverine.

Extension of this scheme to all the alkaloids and reagents reveals certain inconsistencies, mostly due to oxidation phenomena, but these disappear if the alkaloids are arranged in six groups, into which many (probably most) of the amines also fall. By means of this arrangement, which is fully described, it is possible, in combination with microscopic tests, to identify 0.005 grm. of any pure alkaloid. Moreover, it is usually possible to tell if a certain alkaloid is detectable in presence of another or if the two can be separated by precipitation, and, if so, with which reagents.

T. H. P.

**Determination of Nicotine in Commercial Solutions and Insecticides.** A. Sabatié. (*Ann. Falsificat.*, 1930, 23, 544-547.)—Nicotine may be conveniently

determined polarimetrically, pyridine and similar bases, which are sometimes used as adulterants, being optically inactive. Twenty-five c.c. of the commercial solution are diluted to 100 c.c., and 25 c.c. of the diluted solution transferred to a long-necked flask connected with a condenser and with a steam generator. The nicotine is liberated by addition of 2 c.c. of sodium hydroxide solution (36° Bé.), and is distilled off in a current of steam, which is passed slowly at first in order to obviate frothing. Entrainment of drops of liquid is easily prevented by baffling the end of the delivery tube in the flask with a disc of rubber held in position by two pins stuck into the stopper. The nicotine is carried over in the first fraction of 250 c.c., but further fractions of the same volume are collected and examined polarimetrically in a 50 cm. tube. In solutions with concentrations up to 35 per cent. the specific rotation of nicotine  $[\alpha]_D$  has the value  $-80.2^\circ$ .

The result may be confirmed: (1) by titrating the nicotine solution with 0.1 *N*-acid in presence of methyl red as indicator, and (2) by precipitating the base with potassium silicotungstate in solution acidified with hydrochloric acid, weighing the double salt dried at 120° C., and again weighing the residue of silica and tungstic acid left after calcination. Multiplication of the weight of double salt by 0.1011, or that of the mixed residue by 0.1139, gives the weight of nicotine.

If the nicotine solution is adulterated with pyridine, the results obtained by methods (1) and (2) are higher than that of the polarimetric determination. If ammonia has been added as adulterant, the acidimetric result is higher than the polarimetric and precipitation results.

T. H. P.

**Determination of Picric Acid in Solution.** M. François and L. Seguin. (*J. Pharm. Chim.*, 1930, 132, 433-438.)—In the absence of phenol, the necessary quantity of the liquid under investigation, containing about 5 or 6 cgrms. of picric acid, is made up to 50 c.c. with water, and an excess of ammoniacal copper sulphate solution added (copper sulphate, 10 grms., 50 c.c. of ammonia, made up to 250 c.c. with water) so that the liquid over the precipitate is distinctly blue. (Thirty c.c. are necessary for the precipitation of 0.1 gm. of picric acid.) After standing one hour, the liquid is filtered through two weighed 9 cm. papers, and the precipitate carefully washed with 10 c.c. of dilute (1 : 5) ammonia, dried in air without heating, and weighed. The weight of ammonium copper picrate is multiplied by 0.7563 to give the weight of acid. If desired, the ammonium copper picrate,  $[C_6H_2(NO_2)_3OH]_2CuO \cdot 2NH_3$ , may be converted into insoluble black copper oxide and ammonium picrate by means of hot water, the filtrate evaporated, and the residue of crystalline ammonium picrate weighed. In the presence of phenol, the same procedure may be used if the time of contact between the ammonium copper picrate precipitate and the mother liquor is limited to one hour, or the phenol may be eliminated by first adding 1 gm. of crystallised sodium carbonate, so that insoluble sodium picrate is formed, after which the phenol is removed by four extractions with chloroform (20 c.c. each time), and the picric solution is warmed to expel any chloroform, and filtered, and the determination continued as before. In the presence of complex materials such as bread, the liquid portion is filtered

off, the solids washed, and to 100 c.c. are added 5 grms. of white wool in small pieces, and after 24 hours another 5 grms., and so on, until 20 grms. have been used, when all the picric acid will have become fixed. After washing with water, the wool is extracted with ammoniacal alcohol (ammonia 22 per cent., 5 c.c.; alcohol 95 per cent., 50 c.c.; and water 45 c.c.). The resulting ammonium picrate dissolves in the liquid, and after the alcohol has been expelled, the picric acid is precipitated with ammoniacal copper sulphate as described above. D. G. H.

**Reactions of Antipyrin and Pyramidone. Determination of Antipyrin in Pyramidone.** M. Ribere. (*J. Pharm. Chim.*, 1930, 122, 444-446.)—To 2 c.c. of 1 per cent. aqueous solutions of (a) antipyrin, and (b) pyramidone 12 drops of the reagent to be used are added. With 20 per cent. trichloroacetic acid a white precipitate soluble in excess or on heating is formed with (a) and the reaction is negative with (b). Perchloric acid gives similar results. Ammoniacal silver nitrate is immediately reduced by (b) on warming, but not with (a). Six drops of acid mercuric nitrate solution (prepared by dissolving 2 grms. of mercury in 20 grms. nitric acid, and shaking until almost complete disappearance of nitric fumes), gives with (a) a white cloudiness, increasing to an abundant precipitate, transformed on shaking into a greenish coagulate, which dissolves on boiling and reappears, of a brick red colour, on cooling. A violet oxidation colour is formed with (b). If 20 per cent. trichloroacetic acid is added to this acid mercuric nitrate reagent, as little as 0.1 per cent. antipyrin may be detected in pyramidone. One-tenth of a grm. of the suspected material is dissolved in 10 c.c. of water, and to 2 c.c. 6 drops of reagent are added. For over 25 per cent. of antipyrin the reaction is the same as for 100 per cent. Up to 15 per cent. the light white precipitate dissolves on heating to an orange yellow solution, depositing on cooling a slight brick red precipitate; for 10 per cent. a red coloration is obtained on warming, and for 1 per cent., 1 c.c. of reagent is introduced without mixing to 2 c.c. of the solution, and at the junction of the liquids a violet ring (due to oxidation of the pyramidone) is surmounted by a white cloudiness rapidly attaining 1 cm. in height. The limit for the test is about 0.1 per cent. D. G. H.

## Biochemical.

**Plasma Phosphatase. I. Method of Determination. Some Properties of the Enzyme.** H. D. Kay. (*J. Biol. Chem.*, 1930, 89, 235-247.)—The phosphatase of the blood, and particularly that of the blood plasma, has heretofore been relatively little investigated. Preliminary experiments by the author showed that normal plasma contained a weak glycerophosphatase active over a fairly wide pH range from 7.0 to above 9.0. To compare one plasma with another it was necessary to evolve a reliable quantitative method for the determination of this enzyme, and details are given of a simple method which has been devised for the determination of the phosphatase content of the blood plasma of man and of other animals. After many trials to work out a method it was decided not to endeavour

to determine the activity at the optimum hydrogen ion concentration, but to use separated plasma at its natural  $pH$  (of 7.5 to 7.6) plus substrate at about the same  $pH$ , to determine the actual  $pH$  at which the hydrolysis proceeded, and to calculate, from the amount of free phosphate actually liberated, the amount that would have been produced had the reaction proceeded at exactly  $pH$  7.6, all the  $pH$  determinations being made at room temperature. A plasma sample containing 1 unit of phosphatase per c.c. is one of which 1 c.c. will liberate 1 mgrm. of inorganic phosphate (expressed as P) from excess sodium  $\beta$ -glycerophosphate solution in 48 hours at  $38^{\circ}C.$ , and a  $pH$  of 7.6, the hydrolysis to be conducted in absence of added buffer, the  $pH$  determinations to be made at room temperature, and other conditions as stated. In normal plasma the amount of the enzyme per unit quantity of plasma is small compared with the amount present in an equal weight of tissues such as kidney, intestine, or bone. In a determination of the phosphatase content of plasma it is necessary to avoid both haemolysis and the presence of leucocytes in the plasma, since both red and white cells contain more phosphatase than plasma. Certain properties of the plasma enzyme are described. It appears to have the same optimum  $pH$  for activity as that of the phosphatases of other mammalian tissues ( $pH$  8.8 to 9.2). Magnesium ions act as a powerful stimulant to its activity, with an optimum  $pMg.$  between 1 and 2. Calcium ions act as a mild inhibitor. The enzyme will hydrolyse all the phosphoric esters which have so far been presented to it, namely, hexosediphosphate, synthetic  $\alpha$ - and  $\beta$ -glycerophosphates, pyrophosphate, glycerophosphate and guanine nucleotide. The hydrolysis of each of these substrates, with the possible exception of hexosediphosphate, is stimulated by magnesium ions. In young normal children, and in young animals generally, the blood plasma has a higher phosphatase content than in the adult.

P. H. P.

**Gasometric Determination of Cysteine and Cystine.** H. D. Baernstein. (*J. Biol. Chem.*, 1930, 89, 125-131.)—A method for the determination of cysteine and cystine is described which depends upon the reducing properties of the sulphhydryl group of cysteine when oxidised in acid (2 *N*) by a solution of iodine in potassium iodide. Cystine may be reduced quantitatively by nascent hydrogen to cysteine, which is then oxidised back to cystine. A known excess of iodine is allowed to react with the cysteine, and the excess is determined gasometrically by a hydrazine titration in the apparatus of Van Slyke and Neill. Nascent hydrogen evolved from Devarda's alloy in acid solution appears to be an ideal reducing agent for cystine. Under the conditions described pure cystine is quantitatively reduced to cysteine in 10 minutes, and the excess alloy is then filtered off without difficulty. A series of preliminary experiments established the fact that an exact relationship exists between iodine taken and nitrogen liberated from hydrazine in the gasometric apparatus. It is believed that any improvement in the method of cystine assay is of real value because of the importance of cystine as an essential constituent of food and protoplasm, its probable rôle in biological oxidation, and its relation to glutathione and perhaps insulin. The method

described avoids the danger of destruction incident to alkaline hydrolysis, and the errors due to solubility in the isolation and the weighing of the amino acid itself, and is applicable to the determination of cystine in pure proteins and to its liberation during protein hydrolysis. The method is simple, rapid and accurate to about 4 per cent. A mixture which contained 2.31 mgrms. of cysteine and 1.51 mgrms. of cystine gave on analysis 2.32 and 1.52 mgrms. respectively.

P. H. P.

**Saponin from Spinach.** O. Dafert. (*Z. Unters. Lebensm.*, 1930, 60, 408-409.)—Very little saponin occurs in the leaf of spinach, and none in the stalk and seed. The haemolytic index of the root, due to its saponin content, is 1:1000 for sun-dried roots, and 1:400 if dried in an oven at 100° C. It rises from 1:85 for a plant 3 weeks old to a maximum of 1:800 after 7 weeks, and then falls to 1:280 after 20 weeks. The saponin is extracted from the scraped, sun-dried root in a 5-fold volume of 70 per cent. alcohol for 2 hours, followed by extraction for 1 hour with a 2-fold volume of alcohol. The extracts are filtered, the residue pressed, and the crude saponin then separates from the filtrate after 1 day in the ice-chest. It is filtered off, washed with absolute alcohol, the filtrate evaporated to one-third of its volume, cooled, and an additional yield of saponin is thus obtained. The total quantity is dried, extracted with ethyl acetate, and the saponin recrystallised from hot 96 per cent. alcohol in pointed white needles ( $C_{42}H_{78}O_{24}$ , haemolytic index 1:100,000, ash 1.2 per cent., m.pt. 260° to 262° C.). It is readily soluble in dilute methyl or ethyl alcohols or in alkalis or hot water, but is insoluble in chloroform, ether, petroleum spirit, benzene, xylene, acetone, or amyl alcohol. No second saponin was obtained. Hydrolysis with sulphuric acid in 50 per cent. alcohol, and treatment with charcoal gave a white amorphous sapogenin ( $C_{24}H_{44}O_7$ , m.pt. 208° to 212° C.), soluble in alcohols or in ethyl acetate, and insoluble in water, ether or benzene.

J. G.

**Toxic Effect of Fish Liver Oils and the Action of Vitamin B.** E. R. Norris and A. E. Church. (*J. Biol. Chem.*, 1930, 89, 437-449.)—Three years ago in the biological assay of fish liver oils for vitamin A, it was noticed that relatively small amounts of some fish liver oils (less than 2 per cent. of the diet) had a very deleterious effect, and a systematic study was initiated to determine what factor or factors of the oil were toxic or injurious. Previously the toxic effects of cod-liver oil had been noticed, and efforts had been made to correlate the effect with hypervitaminosis of one of the fat-soluble vitamins, the excess of free fatty acids, etc., but the published results are very contradictory. The results of the present study show that some cod-liver oils produce symptoms similar to vitamin B deficiency when given in relatively large amounts to animals (rats) on a diet containing as high as 10 per cent. of yeast. The toxic effect of excess of cod-liver oil can be counteracted by giving large amounts of yeast. The toxic effect of large doses of some cod-liver oils is not due to hypervitaminosis caused by excess of vitamins A or D. Continued small doses of *iso*-amylamine in amounts

which may be found in cod-liver oil produce paralysis, convulsions and lack of growth; the toxic effect may be prevented or cured by added yeast. Continued small doses of choline also produce symptoms identical with vitamin *B* deficiency, which may be prevented or cured by added yeast. The quantitative determination of vitamin *A* in cod-liver oil is influenced by the amount of yeast in the basal diet, even when there is sufficient yeast to satisfy the vitamin *B* requirements under some conditions. It is pointed out that determinations of vitamin *B* by methods of increase in rat weight, where a cod-liver oil of unknown toxicity is incorporated in the diet, cannot be compared. These results might explain some of the discrepancies and variations in both vitamin *A* and *B* determinations. P. H. P.

**Study of the Antimony Trichloride Colour Reaction for Vitamin A.**  
**III. Effect of Concentration of Reagent Used, and the Stability of the Chromogenic Substance to Light.** E. R. Norris and A. E. Church. (*J. Biol. Chem.*, 1930, **89**, 421-435.)—The effect of concentration of the antimony trichloride reagent upon the colour produced with the chromogenic substance of cod-liver oil has been shown. At low colour values the variation of the observed colour with concentration of antimony trichloride is slight. With high concentrations of oil the rate of fading of the colour produced, and consequently the observed colour at 30 seconds is dependent upon the light used for measurement of the colour. Experiments were made to determine which wave-lengths of the visible spectrum were most destructive to the chromogenic substance of cod-liver oil. The results showed that the chromogenic substance of cod-liver oil or vitamin *A* is rapidly destroyed by light of wave-length less than  $500m\mu$ , and practically unaffected by light of wave-length greater than  $500m\mu$ . Tables and graphs show the results. It has also been shown that the presence of oxygen increases the rate of destruction of vitamin *A* by light. P. H. P.

**Antineuritic Vitamin.** A. G. Van Veen. (*Rec. Trav. Chim. Pays-Bas*, 1930, **49**, 1178-1179.)—By suitable treatment of yeast or rice-bran extracts with aromatic acid chlorides or, better, sulphochlorides, it is possible to remove many physiologically active and other substances without destroying the antineuritic vitamin, which, when it has been purified to a certain degree, may be obtained crystalline by utilising the slight solubility of its double salt with platinum or cadmium chloride. By this procedure, 75 kilos. of rice-bran yield, in a short time, about 140 mgrms. of crystalline, but not quite pure vitamin, which is readily purified further by recrystallisation. The product thus obtained responds only faintly to Pauly's reaction with diazotised sulphanilic acid, and, if crystallisation is repeated, the coloration given is scarcely distinguishable from that of the control, although the activity of the vitamin is equal to that of Jansen and Donath's preparation. T. H. P.

**Stability of Vitamin G as Measured by its Growth-Stimulating Effect.**  
N. B. Guarrant and W. D. Salmon. (*J. Biol. Chem.*, 1930, **89**, 199-211.)—Water-soluble vitamin *B* is composed of at least two factors, one of which, the

so-called growth factor, was thought from early work to be more resistant to destruction or inactivation by heat than the antineuritic factor. During attempts to concentrate and purify this supposedly stable factor numerous irregularities were met which seemed to show that destruction or inactivation had taken place during the treatment. The authors have, therefore, investigated the effect of heat, hydrogen ion concentration, oxidation, reduction, de-amination and ultra-violet irradiation upon the growth-promoting activity of vitamin G in yeast or yeast extract. Young albino rats were used for the tests. The results show that yeast and yeast extracts, autoclaved for 4 hours at 20 pounds, lost some of their growth-stimulating effect. Dry yeast heated for 4 hours at 120° C. showed a similar loss of activity. The destruction of vitamin G in yeast extracts by autoclaving apparently depends upon the pH of the extract when autoclaved. Autoclaving of alkaline extracts results in a greater destruction than a similar treatment to an acidic extract. The passing of gaseous oxygen through a hot concentrated yeast extract for 4 hours had very little effect upon its growth-stimulating qualities. The passing of hydrogen sulphide gas through a hot concentrated yeast extract for 4 hours had no effect upon its growth qualities. Whether partial deamination of yeast extracts decreased their growth-promoting effect seemed to depend upon side reactions, rather than upon deamination of the free amino groups. Yeast extracts concentrated to a thick syrupy consistency and irradiated at a distance of 20 cm. from a mercury arc showed some decrease in growth-stimulating effect. The total destruction was greater in alkaline than in acid solution, the result apparently being the combined effect of the separate action of alkali and irradiation.

P. H. P.

## Bacteriological.

**Acido-proteolytic Bacteria in Pasteurised Milk.** C. Gorini. (*Compt. rend.*, 1930, 191, 885-886.)—In the course of the control of pasteurised milk during last summer at the central dairy of Milan, it was noticed that the bottles kept at 22-30° C. showed varying behaviour. Whereas, in most cases, alteration of the milk occurred only after 48-54 hours, with formation of a firm, compact curd and an acidity of 5-7 per 1000, in other instances change took place after 28-34 hours, a soft, flocculent curd and lactic acidity of 2-2.5 per 1000 being developed. The firm curds contained a preponderance of lactic organisms and acido-proteolytic coccus forms, whereas the soft ones showed principally acido-proteolytic bacilli, which are facultative thermophilic organisms of the *Subtilis* group. The latter are among the most injurious micro-organisms to pasteurised milk and, in order to prevent their development during the cooling subsequent to pasteurisation, it is necessary to effect this cooling to below 10° C. as rapidly as possible. T. H. P.

**Testing of Wood Preservatives.** (*Nature*, 1930, 126, 921-922.)—The following conclusions were arrived at by the conference held in June, 1930, at the Berlin-Dahlem Biologische Reichsanstalt, at which Austria, Denmark, Germany, Great Britain, Holland, Japan, Norway, Switzerland, and the U.S.A.



were represented, but these were regarded as in no way final, and a working committee was set up. The effectiveness of a wood preservative against fungi may be suitably estimated by the wood-block method, using Kolle flasks, and the inhibition point should be expressed as kilos. of preservative per cb. metre of wood. The agar method is only to be regarded as of value for preliminary investigation of a new material, and the inhibition point is the interval between that concentration of the material under test in the medium at which growth just occurs, and that at which it is prevented. The wood should be impregnated with two fungi, one of which should be *Coniophora cerebella*, whilst the exact species of the other has still to be selected. The fungi should be chosen after consideration of the use to which the timber is eventually to be put, and the choice will also obviously depend upon the species of wood used (frondose or coniferous). If the values are to be internationally comparable, different research institutes must use the same strains of fungi of known origin. The determination of the toxicity to fungi of a material must be supplemented by investigation into susceptibility to leaching and physical and chemical stability. Tests against spore infection are as important as tests against infection by mycelium. D. G. H.

## Agricultural.

**Colorimetric Determination of Phosphoric Acid in Hydrochloric Acid and Citric Acid Extracts of Soils.** R. G. Warren and A. J. Pugh. (*J. Agric. Sci.*, 1930, 20, 532-540.)—The soil is powdered (1 mm. sieve), 20 grms. boiled in 70 c.c. of hydrochloric acid, and the mixture allowed to stand on the water-bath for 48 hours, cooled, diluted, filtered and the filtrate diluted to 250 c.c. An aliquot (15 c.c.) portion is then heated on a sand-bath for 15 minutes with 0.5 c.c. (2 c.c. for peaty soils) of 20 per cent. sodium permanganate solution to oxidise organic matter, the liquid diluted to 30 c.c., 6 c.c. of 10 per cent. potassium ferrocyanide and 5 c.c. of 10 per cent. manganese sulphate solutions added, followed by dilute (1:1) ammonia until the blue colour changes to purple ( $pH$  6.8 to 6.9). After the addition of 3.5 c.c. of 2 *N* sulphuric acid the mixture is diluted to 100 c.c., filtered, and aliquot portions taken for the actual determination. This procedure removes the iron and produces the optimum conditions for the phosphate reaction. If less than 0.02 per cent. of acid-soluble phosphoric anhydride is present, the oxidised solution should be evaporated, the residue dissolved in dilute acid, and the iron precipitated without removal of the silica. The factor 0.995 corrects for the volume of the ferrocyanide precipitate. Oxidising agents (*e.g.* ferricyanides) impart a colour to the solution and must be absent. For citric acid extracts 25 grms. of soil are shaken for 24 hours with 250 c.c. of 1 per cent. citric acid solution (plus sufficient acid to neutralise any calcium carbonate), and 75 c.c. of the filtered extract oxidised as above with 10 c.c. of hydrochloric acid and 12 c.c. of permanganate solution for 30 minutes, and heated with agitation for a further 30 minutes till no manganese precipitate remains. Iron is removed as before, using 4 c.c. of ferrocyanide and 1.5 c.c. of sulphuric acid solutions. For the actual determination

(after Fiske-Subbarow, *ANALYST*, 1926, 51, 205) 10 to 50 c.c. of extract are diluted to 75 c.c., 10 c.c. of ammonium molybdate solution (25 grms. per litre of 0.5 *N* sulphuric acid) added, and then 4 c.c. of a solution of 0.5 gm. of aminonaphthol sulphonic acid—1:2:4 in 195 c.c. of 15 per cent. sodium bisulphite solution, to which 20 per cent. sodium sulphite solution is added till solution is complete. The mixture is shaken well, diluted to 100 c.c., and the colour matched after 15 minutes against that produced from 2 to 15 c.c. of a standard solution of potassium dihydrogen phosphate (1 c.c.  $\equiv$  0.0001 gm.  $P_2O_5$ ) under the same conditions. The method of Denigès (*ANALYST*, 1921, 46, 460) may also be used, when 1 to 25 c.c. of extract are diluted to 90 c.c. and shaken with 1 c.c. of a mixture of 100 c.c. of 10 per cent. ammonium molybdate solution with 300 c.c. of dilute (1:1) sulphuric acid (stored in the dark), and 3 drops of stannous chloride solution freshly prepared by the action of 0.1 gm. of tin-foil and 1 drop of 4 per cent. copper sulphate solution, in 2 c.c. of hydrochloric acid, the mixture being diluted to 10 c.c. and filtered when reaction is complete. Comparison with 5 to 50 c.c. of standard treated similarly is made after 5 minutes in a volume of 100 c.c. Good agreement with the results given by the gravimetric method were obtained for a variety of soils, and the method overcomes the interfering effect on the colorimetric method of iron in clay soils. If a colorimeter is used, the deeper colours are preferable, with a colour ratio below 1:3, whilst with Nessler cylinders 25 c.c. and 4 c.c. of standard are the maximum amounts which may be used in the Denigès and Fiske-Subbarow methods, respectively.

J. G.

## Organic Analysis.

**Determination of Ethylenic Nitriles.** G. Heim. (*Bull. Soc. Chim. Belg.*, 1930, 39, 458–461.)—The conditions determining selective bromination of  $\beta$ - $\gamma$ -olefinic nitriles in the presence of their  $\alpha$ - $\beta$ -(unsaturated) isomers depend on the nature and concentration of the bromine and nitrile solutions (*i.e.* on the partition-coefficient of bromine between water and the immiscible solvent used), and on the time of reaction. The optimum conditions are obtained with 5 c.c. of a solution of the nitrile, in a suitable solvent, corresponding with 0.025 mol. per 100 c.c., and twice the theoretical quantity of 10 per cent. aqueous potassium bromide solution. The mixture is emulsified by shaking for 10 seconds, a 10 per cent. solution of potassium iodide added, and the displaced iodine titrated with 0.1 *N* sodium thiosulphate solution. Under these conditions the absorption of bromine by vinyl acetic nitrile was almost quantitative (99.8 per cent.), whilst  $\alpha$ - $\beta$ -nitriles (*cis*- and *trans*-crotonic nitriles and acrylic nitrile, and their homologues) absorbed only 0.9 to 3.0 per cent. The compositions of artificial mixtures of the two types of nitrile were determined with a mean error of 0.5 per cent. The crotonic nitriles absorb less bromine than iodine, vinyl acetic nitrile more (*cf.* Linstead and others, *id.*, 1929, 38, 1269, 2139).

J. G.

**New Colour Reaction of Aromatic Amines.** I. De Paolini. (*Gazz. Chim. Ital.*, 1930, 60, 859–862.)—Addition of an aromatic amino compound to an alcoholic suspension of benzoyl peroxide gives, in the cold, but more rapidly on

gentle heating, a coloration which is usually reddish-brown or violet. This reaction, which is given by a considerable number of aromatic amines, substituted amines, and amino-acids, is due to the formation of quinonoid compounds, and it seems probable that the benzoyl peroxide gives rise first to perbenzoic acid.

T. H. P.

#### **The Biuret Reaction. II. The Biuret Reaction of Di-acid Amides.**

**M. M. Rising, J. S. Hicks and G. A. Moerke.** (*J. Biol. Chem.*, 1930, **89**, 1-25.)—Recently, Rising and Johnson (*J. Biol. Chem.*, 1928, **80**, 709) described a study of the biuret reaction of acid imides; the work was an early part of an extensive investigation of the chemistry of the biuret reaction, the results of which, it is hoped, may uncover clues relating to the molecular structure of the proteins, which so characteristically form coloured products with the biuret reagents. Further work upon the chemistry of the biuret reaction is now reported. A discussion is given of the biuret reaction of the typical di-acid amides biuret, malonamide, and oxamide. The sodium copper salts of the first two of these were isolated and analysed, empirical formulae for them deduced, and structures proposed. The biuret reaction is also discussed of the substituted di-acid amides *N*-monoethyl-malonamide, monoethyloxamide, and symmetrical diethyloxamide. Their sodium copper salts were isolated and analysed, empirical formulae deduced, and structures proposed. Schiff (*Ann. Chem.*, 1898, **299**, 257) stated that symmetrical diethyloxamide does not react with the biuret reagents; but when precautions were taken to prevent hydrolysis by the use of a nearly non-aqueous reaction medium containing an excess of alkali and of diethyloxamide, the biuret reaction was found to occur. Biuret, oxamide and malonamide readily show the reaction; mono- and symmetrical di-alkylated oxamides show the reaction, but unsymmetrical di-alkylated and tri-alkylated oxamides do not; *N*-mono-alkylated malonamides show the reaction, whilst further alkylation of the malonamide molecule prevents it. These findings aid in the prediction, with some degree of accuracy, of the behaviour of di-acid amides toward the biuret reagents. The theory of the biuret reaction is extended with regard to the atoms concerned in the biuret reaction of di-acid amides. A molecule which is sensitive to the biuret reaction is both an acid and a base, and the reaction of such a molecule with alkali and cupric ion involves salt and complex ion formation. Apparently 4 ionisable hydrogen atoms take part in the reaction. A typical acid imide molecule contains 1 such hydrogen atom, and hence 4 imide molecules are to be found in the biuret reaction product of an acid imide, whilst a di-acid amide with 2 ionisable hydrogen atoms forms a product containing only 2 amide molecules. The bare essentials in the matter of atoms for the occurrence of the biuret reaction of a di-acid amide seem, therefore, to be:—(1) Two acid hydrogen atoms in each molecule for salt formation, and (2) one or more amine nitrogen atoms in each molecule for complex ion formation. Apart from the "essential atoms," several factors influence the behaviour of the di-acid amides toward the biuret reagents; one of these may be termed the ionising power of the amide as an acid. If the delicate balance between acidity

and basicity in the amide molecule is disturbed, the reaction may not occur. In the oxamide series the authors have correlated the progressive decrease in acid strength attendant upon alkylation with decreased tendency to show the biuret reaction, and it is shown that the occurrence of the biuret reaction is prevented earlier in the biuret and malonamide, than in the oxamide, series. An explanation is offered for the inhibition of the biuret reaction of di-acid amides by multiple substitution of alkyl groups in their molecules, and by separation of the amide groups by carbon or nitrogen atoms.

P. H. P.

**Determination of Starch in Finished Goods and Yarns.** D. A. Derrett-Smith. (*J. Text. Inst.*, 1930, **21**, 583r.)—The cloth is heated with dilute sulphuric acid for  $2\frac{1}{2}$  hours, the liquid filtered, the filtrate neutralised and treated with an alkaline solution of copper, and the resulting cuprous oxide washed and dissolved in a standard solution of ferric ammonium sulphate. This is then titrated with weak standard potassium permanganate solution, the amount of starch originally present being then calculated from the number of c.c. required. Eleven determinations may be carried out by this method in 3 to 4 hours.

The details of the method are as follows: The weight of cloth to be taken should be such as to correspond to a titre not exceeding 35 c.c. of  $N/25$  potassium permanganate solution. (In the case of a full-bleach cloth with medium starching, 1 gram. is suitable.) It is cut up into squares of about  $\frac{1}{8}$  inch, or in the case of yarn, into lengths of  $\frac{1}{4}$  inch, and placed in a dry 175 c.c. conical flask, having a loose glass pear stopper. Thirty c.c. of water are added, followed by 25 c.c. of  $2N$  sulphuric acid. The flask is then immersed for exactly  $2\frac{1}{2}$  hours in a boiling water-bath kept at constant level. The material is filtered off by suction through a sintered glass funnel, the fabric transferred to the funnel, and the flask rinsed twice with 5 c.c. The solution is made nearly neutral to methyl orange by adding slowly about 22.5 c.c. of  $2N$  sodium carbonate solution, is then heated to boiling and treated with a boiling solution of 95 c.c. of alkaline copper solution (sodium carbonate, anhydrous, 129.7 grms.; sodium bicarbonate, 50 grms.; water to 1000 c.c.) and 5 c.c. of copper sulphate solution (100 grms. in 1000 c.c.), the flask then being immersed in the boiling water bath for half-an-hour. On to the filtered and washed cuprous oxide are poured two successive portions of 15 c.c. of the iron sulphate solution, followed by two washings with  $2N$  sulphuric acid. The filtrate is then titrated direct with  $N/25$  potassium permanganate solution. In all determinations a "blank" test should be carried out simultaneously on the fabric before starching. If 1 gram. of fabric is taken, 11.36 c.c. of  $N/25$  permanganate are equivalent to 0.99 per cent. of starch on the dry starched fabric containing 6 per cent. of moisture. The method can also be applied to grey, boiled and bleached flax yarns. R. F. I.

## Inorganic Analysis.

**Analytical Research on Protargol (Determination of Alkalinity and Silver.** A. L. Dragenesco and E. Weinberg-Sachetti. (*J. Pharm. Chim.*, 1930, **12**, 536–539.)—*Alkalinity.*—Protargol (0.25 gram.) is dissolved in 50 c.c. of

water, and 1 grm. of sodium thiosulphate (neutral) is dissolved in the liquid which is then titrated with *N*/10 sulphuric acid, using phenolphthalein as indicator, until the colour matches that of a similar solution which does not contain phenolphthalein. The function of the thiosulphate is to change the deep brown colour of protargol in water to light yellow. *Silver*.—Silver is determined by titration with *N*/10 thiocyanate, the organic matter having been destroyed either by calcination or by wet oxidation with sulphuric acid and permanganate; the latter method gave the more consistent results. S. G. C.

**New Method for the Determination of Mercury in Mercuric Cyanide.**

**E. Cattelain.** (*J. Pharm. Chim.*, 1930, 12, 529–531.)—Mercuric cyanide is immediately reduced in dilute hydrochloric acid solution by a mixture of hydrogen peroxide and hypophosphorous acid, giving metallic mercury. In this respect it has been found to differ markedly from other salts of mercury, which, under the same conditions, yield mercurous chloride, as shown by Vanino and Treubert (*Ber.*, 1897, 30, 1999). In order to obtain mercurous chloride from mercuric cyanide by reduction with hypophosphorous acid and hydrogen peroxide, it is first necessary to decompose the cyanide radicle. The method given is as follows:—To the mercuric cyanide solution (10 c.c.) is added sodium chloride solution (10 c.c., 10 per cent.), ammonia (2 c.c., sp. gr. 0.925), and saturated potassium permanganate (10 c.c., a large excess is stated as being necessary). The solution is maintained at 80° C. for half-an-hour. The excess of permanganate is then reduced by adding ethyl alcohol (2 c.c.) and heating for 5 minutes; the heating is continued for another 5 minutes after adding concentrated hydrochloric acid (5 c.c.) to dissolve the manganic hydroxide produced. The solution is cooled. Hydrogen peroxide (20 c.c., 10–12 vol.) and hypophosphorous acid (2 c.c.; 50 per cent., sp. gr. 1.274) are added, and, after the lapse of 5 minutes, the precipitate of mercurous chloride is filtered off on a Jena sintered-glass crucible (1 G4), washed with cold water, and dried at 100° C. The process is supported by one test result. S. G. C.

**“Direct Green-B.” A New Sensitive Reagent for Copper.** **P. Sisley and David.** (*Bull. Soc. Chim.*, 1930, 47–48, 1188–1192.)—The solution to be tested is brought to pH 6 to 9, and 0.1 to 10 c.c. of a 0.1 per cent. solution of direct green-B (No. 593, *Colour Index*, cf. *Allen's Organic Analysis*, Vol. VI, p. 200) added to 25 c.c. It is convenient to work with 4 pairs of 25 c.c. test-tubes containing 0.1, 0.5, 2.6, and 10 c.c. of the dye solution, respectively, in order to obtain different ratios of dye to copper. One tube of each pair is heated for 15 minutes on the water-bath, and the change in colour from blue-green to violet-rose is compared with that obtained from a solution containing a known amount of copper sulphate, the tube corresponding with the greatest concentration of dye and a ratio of dye to copper within the range 5 to 50 being selected for the comparison. This gives an estimate of the copper present, while the unheated portion serves as a control. With the optimum ratio (10:1), 0.1 mgrm. of copper per litre is detectable. The reaction proceeds slowly in the cold, and is inhibited by acid, but is unaffected

by the nature of the anion. Iron, chromium and mercury precipitate the dye, without, however, influencing the colour-change, and silver gives a brown-yellow colour. Neutral salts (1 to 10 per cent.) which diminish ionisation, retard the reaction, and in the presence of organic colloids which mask the reaction, the solution should be evaporated and the ash tested. Water distilled in copper apparatus was found to give a positive reaction. J. G.

**Separation of Lead as Chromate from Mercury and from Copper.** H. Fink and J. Schormuller. (*Z. anal. Chem.*, 1930, 82, 361-365.)—From *Mercuric ion*.—The faintly acid solution is treated with an adequate excess of sodium acetate and 50 c.c. of 5 per cent. sodium chloride solution, boiled, and precipitated with 5 c.c. of hot 5 per cent. dichromate solution (bulk, 100 c.c.). When cold, it is filtered through a porous porcelain crucible for gravimetric, or paper for iodimetric, work; the precipitate is washed with cold, 1 to 2 per cent. acetic acid. The filtrate is acidified with hydrochloric acid and heated with alcohol, cooled, and treated with hydrogen sulphide; the mercury is determined as sulphide. From *Mercurous ion*.—The solution, treated with sodium chloride as in the preceding case, is stirred and treated with bromine-water until the mercurous chloride has re-dissolved. After addition of sodium acetate, the above process is applied. From *Copper*.—The method has been used by Hodgson for the determination of lead in brass and like alloys (*Chem. News*, 1919, 118, 37). The procedure given above can be used, the addition of sodium chloride being unnecessary. W. R. S.

**New Method for the Separation of Nickel and Cobalt.** G. Schuster. (*Ann. Falsificat.*, 1930, 32, 485-487.)—Cobalt and nickel are precipitated by sodium hydroxide and hydrogen peroxide as cobaltic hydroxide and nickelous hydroxide, respectively; it is stated that the nickel hydroxide can be dissolved from the mixed precipitate in an ammoniacal solution of ammonium chloride. In the one quantitative test experiment described, sodium hydroxide (3.5 c.c., 10 per cent.) and hydrogen peroxide (2 c.c., 10-12 vol.) were added successively to the boiling neutral solution of cobalt and nickel sulphate (50 c.c.) and the precipitate filtered and washed with water. The precipitate was digested for 10 minutes on the filter with 30 c.c. of the ammoniacal ammonium chloride solution (ammonia (22° Bé) 20 grms.; ammonium chloride, 10 grms.; water not stated), the outlet of the funnel meanwhile being closed by a rubber cap. The residual cobalt hydroxide was washed with 10 c.c. of the ammoniacal liquid and the cobalt determined by reduction in hydrogen after calcination and also by the  $\alpha$ -nitroso- $\beta$ -naphthol method. The nickel in the filtrate was determined gravimetrically by means of dimethylglyoxime.

S. G. C.

**Rapid Method for the Determination of Selenium.** E. Benesch and E. Erdheim. (*Chem. Ztg.*, 1930, 54, 954.)—The solution, in which the selenium may be present either as selenious or selenic acid, is diluted to about 400 c.c., acidified with hydrochloric acid (about 5 c.c.), and a very large excess (30 grms.) added of hydrazine hydrochloride or hydrazine sulphate (preferably the former on

account of its greater solubility). The solution is boiled for half-an-hour, and then kept on a water-bath until the precipitate of selenium, which has changed from the red to the black variety during the boiling, settles out. The precipitate is filtered off on a weighed filter which has been dried at  $110^{\circ}\text{C}$ ., washed with hot water, and the whole dried at  $110^{\circ}\text{C}$ . and weighed. Selenium, which is present in samples in powder form, is brought into solution as follows:—The sample is well mixed, in a nickel crucible, with iron oxide (3 grms.), potassium chlorate (3 grms.) and sodium bicarbonate (4 grms.). The mixture is heated until it sinters and afterwards extracted with water; an aliquot part of the filtered extract is used for the above determination. Details for the recovery of the unused hydrazine are given. The three test results which are given show a satisfactory recovery of selenium, selenic acid or selenium being the starting material. The tests made do not cover the possible influences of other elements. S. G. C.

**Volumetric Determination of Sodium.** A. Blenkinsop. (*J. Agric. Sci.*, 1930, 22, 511–516.)—The solution, containing not more than 12 mgrms. of sodium (preferably as chloride), is evaporated, and 15 c.c. added of a mixture (filtered after 24 hours) of 10 grms. of uranyl acetate ( $2\text{H}_2\text{O}$ ) in 6 c.c. of 30 per cent. acetic acid and 60 c.c. of water with 30 grms. of zinc acetate in 3 c.c. of acetic acid and 32 c.c. of water (*cf.* Barber and Kolthoff, *ANALYST*, 1928, 53, 456). The mixture is stirred, the precipitated  $(\text{UO}_2)_3\text{ZnNa}(\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$  filtered off in a Jena G-4 filter-funnel after 1 hour, and washed 3 times with 2 c.c. of a saturated solution of the precipitate in 95 per cent. alcohol. A solution of the uranyl zinc sodium acetate in 10 c.c. of hydrochloric acid is transferred to a flask, the air removed by means of a current of carbon dioxide, and the uranium reduced by the action of an excess of 0.02 *N* titanous chloride solution for 2 minutes at  $40^{\circ}\text{C}$ . The excess is determined by back-titration with iron-alum solution in the presence of 20 c.c. of 2 per cent. hydrofluoric acid, and 10 c.c. of 10 per cent. potassium thiocyanate solution till a permanent red colour is obtained. It is convenient just to over-run the end-point and to re-titrate again with the titanous chloride solution till the red colour disappears. Then  $1\text{Fe} = 2.133\text{U} = 0.06865\text{Na}$ , or weight of sodium  $\times 0.01495 =$  weight of uranyl salt, or 1 c.c. 0.02 *N*  $\text{TiCl}_3 = 0.0000767\text{Na}$ , the titanium solution being standardised against pure ferrous ammonium sulphate which has been carefully oxidised with potassium permanganate. The hydrofluoric acid stabilises the uranous compound, without affecting the ferric salt, by the precipitation of the amorphous green uranous fluoride. The accuracy is 0.1 mgrm. of sodium, and is unaffected by calcium, magnesium and relatively large amounts of potassium. Iron and aluminium (as phosphates) may be rendered insoluble by ignition without loss of sodium, or may be removed by precipitation with ammonia and ammonium carbonate, and removal of the reagent by volatilisation. J. G.

## Microchemical.

**Tests in Capillary Tubes applied to the Identification of Nitrates and Nitrites.** F. L. Hahn. (*Mikrochem. Emich-Festschrift*, 1930, 143–147.)—

Capillary tubes, 1–2 mm. in diameter and 5–6 cm. long, drawn out at each end to 0.1–0.2 mm. diameter and 1 cm. long, are used for the diazo test for nitrites and the diphenylamine test for nitrates. The reagent is allowed to run up the capillary, so that it fills 0.5–1 cm. of the wider part of the tube, which is then sealed at both ends. A number of capillaries may be filled and kept sealed until they are used, when the tip of the fine capillary at the end containing the liquid is broken off. The tube is warmed above the liquid with a micro flame, so that a small drop of liquid is expelled; this is removed, and the tip of the capillary dipped in the test solution, which is sucked up as the tube cools, when it is re-sealed. The sealed capillary can be heated, if necessary, for the reaction to take place.

**Diphenylamine test.**—The reagent is prepared from 20 mgrms. of a mixture of 1 part of diphenylamine and 20 parts of sodium chloride, in 1 c.c. of water and 2 c.c. of concentrated sulphuric acid. The mixture dissolves on heating. Using 1 c.mm. of the test solution, 0.01% of nitrate can be detected, in a dilution of  $10^{-6}$ .

**Diazo test.**—Using 1 c.mm. of test solution,  $10^{-4}$ % of nitrite can be detected, in a dilution of  $10^{-7}$ . For the test for nitrate by conversion into nitrite similar capillary tubes are used, except that they are drawn out to a finer tip at one end only. A little finely powdered lead formate is added, and the test solution is allowed to run up the fine end when both ends are open. The capillary is then sealed at both ends, heated for 5–15 minutes over a water-bath, when the fine end is opened and the diazo reagent solution added, as before. Using 1 c.mm. of test solution,  $4 \times 10^{-3}$ % of nitrate can be detected, the dilution is  $4 \times 10^{-6}$ .

**Precipitation test for nitrite.**—The reagent used is 2,4-diamino-6-hydroxypyrimidine (Merck's "Rosit"), the free base is used in 2 N acetic acid solution, or the sulphate or chloride is dissolved in a little mineral acid (0.1 gm. per c.c.). Equal volumes of the reagent and test solution are drawn up into the test capillary, with the wider end left unsealed, the sediment is centrifuged into the narrower end, and when no precipitate is visible with the naked eye the microscope is used. Using 1 c.mm. of test solution  $<0.01\%$  of  $\text{NO}_2^-$  can be detected in a dilution of  $<10^{-5}$ .

J. W. B.

### Micro Vacuum Sublimation of Synthetic Compounds used in Medicine.

**R. Eder and W. Haas.** (*Mikrochem., Emich-Festschrift*, 1930, 43–82.)—The different micro vacuum sublimation methods are described and discussed, and for 0.01–0.05 gm. of material Eder's method (*ANALYST*, 1913, **38**, 426) is recommended, working at pressures of 18–12 mm. with an oil or sulphuric acid heating bath. The sublimation should take about 1 hour. For amounts of material of about 0.2 gm., they use Diepolders' method. Using the two methods, the temperature at which sublimation begins and the optimum temperature, and also the microscopic appearance of the sublimate of a large number of substances, were observed. The substances include veronal, adaline, bromural, isopral, neuronal, antipyrin, phenacetin, acetanilide, orthoform, tutocain, salol, salicylic acid, luminal, dial, phanodorm, sandoptal, allylisopropyl-bartitric acid, nirvanol, pyramidol, antifebrin,



lacto-phenin, citrophen, dulcin, saccharin, novocaine, anaesthesin,  $\beta$ -eucain, psicain, stovain, holocain, alypin, salophen, aspirin, and salacetol. The results are summarised in two tables, and photographs are given of the microscopic appearance of crystalline sublimates.  
J. W. B.

**Crystallographic Examination of the Micro Sublimates of Synthetic Compounds used in Medicine.** W. Haas. (*Mikrochem., Emich-Festschrift*, 1930, 83-119.)—The crystalline sublimates of the compounds named in the preceding abstract are examined in detail in ordinary light, and in parallel and converging polarised light. The examination includes the measurement of refractive indices, determination of the optical character, and whether the crystals are mono- or bi-axial, the interference colours and general characteristics. The results are summarised in tabular form.  
J. W. B.

**Microchemical Identification of some Barbituric Acid Compounds.** L. van Itallie and A. J. Steenhauer. (*Mikrochem., Emich-Festschrift*, 1930, 166-169.)—The tests used in the toxicological examination of material for barbituric acid compounds are described. The test substance is dissolved in potassium hydroxide solution, but an excess of alkali is to be avoided. The barbituric acid compound is precipitated with acetic acid, and with ammonium phosphate, and the formation of the crystals is observed. From the various results of the reactions with thallium acetate, ammoniacal silver solution, ammoniacal copper solution, a saturated solution of bromine and water, and baryta water, it is possible to identify the individual compounds. Drawings are given of the different crystals, and the results of the tests are summarised in a table.  
J. W. B.

**Application of Microchemical Methods to the Analysis of Pigments of Paintings.** H. Hetterich. (*Mikrochem., Emich-Festschrift*, 1930, 152-162.)—The micro qualitative methods of Emich, carried out on a microscope slide, together with the "spot" test methods of Feigl, are applied to the rapid accurate analysis of very small samples of pigments in paintings. Details are given of the analysis of the pigments used in the blue, the green and the red portions of an old picture.  
J. W. B.

## Physical Methods, Apparatus, etc.

**New Method for the Optical Determination of Atmospheric Ozone.** A. I. Duninowski. (*Compt. rend.*, 1930, 191, 859-861.)—By measuring the atmospheric absorption in the visible region of the solar spectrum, with the help of a prism spectroscope with equatorial mounting and a photographic registering apparatus, values have been obtained for the thickness of the ozone layer of the air to within about 0.2 mm. The results obtained at Montpellier during the period August-December, 1929, indicated, for this layer, thicknesses varying from 2.0 to 3.6 mm.  
T. H. P.

**Ultra-Violet Absorption of Honeys.** J. Stitz and J. Koczkaś. (*Z. Unters. Lebensm.*, 1930, 60, 420–425.)—The Bunsen-Roscoe absorption extinction coefficient ( $e$ ) is given in terms of the intensities of incident light ( $I_0$ ) and transmitted light ( $I$ ), the concentration ( $c$ ), and the thickness of the layer of solution examined ( $x$ ) by  $(1/cx) \log I_0/I$ . By means of the Judd Lewis sector photometer and the Hilger quartz spectrograph  $e$  was determined for wave-lengths ( $\lambda$ ) of 5500 to 2350 Å from the measured values of  $\log I_0/I$ , with a relative error of  $\pm 2.2$  per cent. A layer 1 to 4 cm. thick was used, the source of illumination being a tungsten iron arc with electrodes 3 mm. apart in front of a 0.02 mm. slit. Absorption curves, in which  $e$  is plotted as ordinate against  $\lambda$ , are shown for 6 varieties of genuine honey, a characteristic curve always being obtained, with a sharp maximum at 2700 Å corresponding with  $e=0.13$  to 0.145 for a 1 cm.-layer of a 4.5 to 6.5 per cent. solution. Since water has almost negligible powers of absorption, addition to honey of more than the amount usually present (14 to 22 per cent.) produces a marked decrease in  $e$ . Glucose, fructose and sucrose give higher, but relatively low, absorption maxima, the value for fructose being the greatest. For commercial sucrose  $e$  is slightly higher than for the pure product. Dextrin, on the other hand, has an extremely high absorption in the same region (*i.e.* 2700 to 2800 Å). Addition to honey of 22 per cent. of a sucrose solution containing 47.2 per cent. of sucrose lowered  $e$  by 0.3. The total absorption of honey cannot be determined from the individual absorptions of its sugar constituents alone, since the nature and concentration of the ash, colouring matter and proteins has a considerable influence. J. G.

## Reviews.

**ELEMENTARY ANALYTICAL CHEMISTRY—QUALITATIVE AND QUANTITATIVE.** By FRANK CLOWES, D.Sc., Lond., and J. BERNARD COLEMAN, A.R.C.S. Revised by FRANCIS ARNALL, Ph.D., M.Sc., F.I.C., and F. N. APPLEYARD, B.Sc., F.I.C. 11th edition. London: J. & A. Churchill. 1930. Price 6s.

Clowes and Coleman's well-known standard book on elementary analytical chemistry, both qualitative and quantitative, has now reached its 11th edition, and has been revised and brought up to date by Dr. Arnall and Mr. Appleyard.

Although, primarily, this book is written for students working for qualifying examinations, the field covered is so large that it might well be used for the higher examinations of the University of London, such as the Intermediate Science and Pharmacy examinations of that University. In this edition considerable new matter has been introduced, mainly in the section on Volumetric Analysis, also more modern methods of group separation have been substituted. Otherwise,

the order of previous editions is generally followed, and comprises five divisions, embodying Qualitative Analysis, Volumetric Analysis, Gravimetric Analysis, Inorganic Preparations, Organic Compounds, together with an Appendix containing a Table of Atomic Weights, and one of Logarithms. Exercises for practice are also given.

The whole conception of the work is well framed, and this also applies to the separate divisions. The work is very free from mistakes, and is presented in a way that a student can readily understand. A large field is covered in a comparatively small space, and this is effected without in any way impairing the value of the work. The student will find this book a real help in his study of elementary analytical chemistry. The revised edition marks a decided advance on previous ones, and should conduce to the continued popularity of an already well-known book.

ERIC VOELCKER.

MIKROCHEMISCHES PRAKTIKUM. By FRIEDRICH EMICH. 2<sup>te</sup> auflage mit einem abschnitt über TUPFELANALYSE, by FRITZ FEIGL. Pp. xii+157, with 83 illustrations. 1931. Munich: J. F. Bergmann. Price M.12.80.

Although there are a number of books on branches of applied micro-chemistry, such as toxicology, analysis of blood, and plant micro-chemistry, comparatively few have been written on the subject as a whole. Prof. Emich has written the only text-books covering the whole range of micro-chemistry, with the exception of organic quantitative work, which is sufficiently described by Pregl in his book "Quantitative Organic Micro-analyses." Prof. Emich's two books, the "Lehrbuch" and the "Praktikum," treat the same subject somewhat similarly, except that the "Lehrbuch" contains more detailed theory, whilst the "Praktikum" is meant for a student's handbook and a book for use in the laboratory. The "Praktikum," which was first published in 1924, has now been brought completely up-to-date, and, as micro-chemistry has advanced considerably in the last few years, the book contains much new matter.

The first part is concerned with apparatus and methods. The microscope and its use is described only briefly, since more detailed descriptions are available in books on chemical microscopy. The apparatus and methods of qualitative analysis are described in detail, with the aid of drawings, so that the apparatus can easily be constructed by anyone with a slight skill in glass blowing, and with the aid of a laboratory mechanic.

Prof. Emich describes the different methods of micro recrystallisation, fractionation, boiling point and melting point, determinations and sublimation, and there is a new section on liquid striation which describes recent work of the author and some of his pupils.

The quantitative inorganic section, which in the new edition is much enlarged, is particularly valuable, as no other text-book deals with this subject. The new

"filter stick" filtration devised by the author is carefully described; this is both the simplest and most accurate method of micro-filtration, which, with a little practice, even those who are not trained chemists can use with success.

The second part of the book consists of the description of 76 practical examples applying the general methods of the first part. These include qualitative tests for all the common inorganic elements and radicles individually, the analysis of mixtures, and the tests for elements in organic compounds. The reactions and some preparations are given of many of the more common organic compounds and groups, and Rast's micro molecular weight determination is described. The quantitative examples include the gravimetric determination of barium, potassium, aluminium, and nickel, and mixtures of calcium and magnesium, and silver and copper, and Pregl's electrometric determination of copper. There is an increasing tendency in inorganic qualitative work to use specific tests, by the "spot" method, and in a section at the end of the book, Dr. Feigl, the leading authority on this method of analysis, describes the method in general, and gives a few examples of the tests, which are usually colour tests, involving the use of an organic reagent.

A very valuable section of the book is the appendix describing the apparatus and where it may be obtained. Three lists are given; the first containing the most essential apparatus, and the other two describing various desirable additions. Much of the apparatus is very simple, and, in the preface, Prof. Emich points out that any chemist who owns a microscope can carry out qualitative micro-chemical tests, and anyone with a micro balance can do the quantitative inorganic work.

The book is written in a clear and interesting style, and contains many illustrations. It is well printed, and the use of small type serves to distinguish the work for more advanced students from the important tests and determinations which every micro-chemist should carry out. As a new and revised edition of Pregl's book has recently been published, it is particularly useful that Prof. Emich's book has also been revised, so that the most recent general work in all branches of micro-chemistry is now available in text-book form.

JANET W. BROWN.

INTRODUCTION TO PHYSIOLOGICAL CHEMISTRY. By MEYER BODANSKY, Ph.D.  
2nd Edition, re-written and re-set. London: Chapman & Hall. 1930.  
Price 20s. net.

This text-book of biochemistry is fairly comprehensive, and the present edition brings it thoroughly up-to-date. Though general in scope, those parts having reference to human physiology are stressed; it is, therefore, more suited to the medical student than to the student of general biochemistry, and would, in fact, form an admirable companion to any of the well-known text-books of physiology.

The style is clear, and complex relationships are explained by the use of excellent diagrams; at the same time the author does not claim finality, and the reader is constantly referred to original authorities.

Among much that is good one criticises apologetically, but the last paragraph on p. 406 embalms a hoary error in attributing the main effect of adrenaline to carbohydrate rather than to fat metabolism, in spite of the work of Cori and Cori, to whom the author nevertheless refers.

Sulphur metabolism receives short shrift, the important contributions of Hele and his co-workers being omitted.

The chapters on nutrition and calorimetry are particularly good.

M. STEPHENSON.

THE SPIRIT OF CHEMISTRY. AN INTRODUCTION TO CHEMISTRY FOR STUDENTS OF THE LIBERAL ARTS. By ALEXANDER FINDLAY, Professor of Chemistry, University of Aberdeen. Pp. xvi+480. Illustrations. London: Longmans, Green & Co. 1930. Price 10s. 6d.

"It is not the particular facts of a science that constitute its vitality, but the generic facts or conceptions to which they have elevated the mind. Facts are the body of science, and the idea of those facts is its spirit." Such is the quotation which Professor Findlay has chosen as the motto for his title-page. It is an excerpt from Samuel Brown's *The History of Science*; it serves as the text for Professor Findlay's short dissertation on "The Aim and Scope of Chemistry," which concludes with the observation that the study of chemistry "is not only a means of cultivating the mind and of training and strengthening the scientific habit of thought, but it also brings us into closer relations with, and gives a fuller understanding of, the physical universe in which we live. In the study of chemistry one must seek not only the knowledge by which material results may be achieved, but the understanding of its laws and theories by which alone intellectual satisfaction can be secured." The author says that his book has been written for the increasingly large number of university students who take chemistry as a subject of study in the prosecution of a scheme for achieving general culture rather than as a branch of professional study. He has written it so as to "appeal to the imagination and intellectual interests of those who are not destined for a scientific career, but who desire to understand something of the intellectual progress of recent years and to gain some knowledge of a branch of science on which much of our present-day civilisation is based."

The work will have a much wider circle of readers than is indicated in the foregoing remarks, because it is cast in a form which will be greatly appreciated by all senior students of chemistry, as well as by those professional chemists who desire to maintain the inspirational energy obtainable by reviewing from time to time the historical development of their special branch of science. By taking the more important aspects of theoretical and practical chemistry for treatment in separate chapters and treating of them severally from the historical point of view, bringing the account of each down to the present day, the author has quickened the interest alike in historical facts and present-day knowledge. An

example will help to show how wide is the net used by Professor Findlay to catch all the material suitable for illuminating his text: Chapter X deals with "the Gases of the Atmosphere and the Phenomenon of Combustion"; it begins with an illustration of ancient Egyptians using the foot-bellows, and it ends with a picture of a neon lighthouse. Interesting biographical notes accompany the information as to the contributions made by all the chief persons who have played their part in the growth of chemistry, and the brilliant achievements of the better known philosophers, whose names are known to everyone, are not allowed to entirely obscure those of men whose discoveries are but rarely referred to in the more popular histories of science. Jean Rey, Stephen Hales, Döbreiner (of Jena), Perrin (of Paris), Thomas Northmore, John Mayow, Daniel Rutherford (the botanist), and many other of the less well-known persons who have contributed to enrich chemistry find their appropriate place in Professor Findlay's presentation of the "Spirit of Chemistry." The interest is greatly increased by the profusion of illustrations; there are over eighty figures in the text, and nearly seventy portraits and other pictures. The general reader and the student will feel grateful for the list of books, given in a selected bibliography, which may be read to augment the matter in each of the twenty-nine chapters. There is an excellent index, and the volume is printed in a manner to sustain the reputation of the publishers. Once again, Professor Findlay has laid his fellow chemists under a debt of gratitude by treating his subject so as to commend their science to all men of education.

WILLIAM KIRKBY.

FÄLSCHUNGEN. Edited by Dr. SIEGFRIED TÜRKEL. Pp. 93. Graz: Ullr. Moser. 1930. Price M.12.

This book, which is one of a series of publications on scientific criminology, consists of a collection of contributions, by several authors, on frauds and forgeries, and is issued under the editorship of Professor Türkkel, of the Police Headquarters in Vienna. Its aim is to direct attention to ways in which spurious articles are prepared for sale, rather than to give full particulars of the methods of detecting the frauds.

The first section, written by Dr. Türkkel, deals with the methods of forging or reconstituting natural history specimens, such as skeletons, minerals, fossils, butterflies, etc. This is followed by a section by Dr. Penn on seals and the methods of tampering with them. It is, perhaps, hardly advisable to give such full working details of how to take copies from seals on documents—even the composition of the most suitable amalgams and alloys for making moulds from the seal is given—and it would have been better to have devoted more space to the methods of detecting the fraud. Reference is made to various chemical and optical methods of examination, including the use of ultra-violet light, but there is no mention of the work of Osborn, who has dealt very fully with the subject in his "Questioned Documents."

Other sections deal with the fraudulent imitation of old book-bindings and drawings, and there is an interesting account of a forged letter of Schubert ingeniously built up by cutting words from reproductions of two genuine letters in a dealer's catalogue, pasting these on a sheet of paper, so as to form a new letter, and then copying this by means of tracing.

The last three sections of the book deal with the examination of oil paintings by microscopical and microchemical methods, with the X-ray photography of pictures (illustrated by a series of interesting plates), and with the identification of paintings. The methods, briefly described and summarised, are, in the main, those with which English chemists have been made familiar by the work of Professor Laurie, to whom, by the way, only the briefest references are made.

The book, as a whole, should prove a useful guide to some of the more obscure paths followed by the forger, but it would gain much by the addition of full bibliographies to all the sections, and it is a blemish to issue a reference work of this type without an index.

EDITOR.

## Publications Received.

THE MICROSCOPIC EXAMINATION OF CATTLE FOODS. By S. T. PARKINSON and W. L. FIELDING. Ashford: Headley Bros. Price 6s. 6d.

PRACTICAL PHYSICAL CHEMISTRY. By A. FINDLAY. 5th Edition. Longmans. Price 7s. 6d. net.

THE MANUFACTURE OF ARTIFICIAL SILK (RAYON). By E. WHEELER. Chapman & Hall. Price 12s. 6d. net.

BACTERIOLOGICAL TECHNIQUE. By J. W. H. EYRE. 3rd Edition. Baillière, Tindall & Cox. Price 21s. net.

QUANTUM CHEMISTRY. By A. HAAS. Translated by L. W. CODD. Constable. Price 6s.

ABRIDGED SCIENTIFIC PUBLICATIONS FROM THE KODAK RESEARCH LABORATORIES. Vol. XIII. Rochester, New York.

THE RELATIVE VALUES OF COD-LIVER OILS FROM VARIOUS SOURCES. By J. C. DRUMMOND and T. P. HILDITCH. (Empire Marketing Board) H.M. Stationery Office. Price 1s. net.

CINCHONA TERCENTENARY CELEBRATION AND EXHIBITION, 1930. The Wellcome Foundation, Ltd.

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, February 4th, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of Cecil Chilvers, B.Sc., F.I.C., Jack Hubert Hamence, M.Sc., A.I.C., Cecil John House, B.Sc., A.R.C.Sc., F.I.C., and Henry George Rees, B.Sc., A.R.C.Sc., A.I.C.

Certificates were read for the second time in favour of Kenneth Bullock, M.Sc., Ph.D., and Frederick Cecil Hymas, B.Sc., A.I.C.

The following were elected Members of the Society:—Arthur Nicholls Ainsworth, B.Sc., Bertram Arthur Gough, William Henry Gough, M.Sc., A.I.C., and William Henry Shilling, B.Sc., A.I.C.

The following papers were read and discussed:—"Some Factors affecting the Solubility of Milk Powder," by L. H. Lampitt, D.Sc., F.I.C., and J. H. Bushill, M.Sc., A.I.C.; i, "The Determination of the Hydroxyl Content of Organic Compounds: Estimation of Castor Oil," ii, "The Determination of the Carbonyl and Aldehyde Content of Organic Compounds: Estimation of Phenylhydrazine," by S. Marks, M.Sc., A.I.C., and R. S. Morrell, Ph.D., F.I.C.; "Food Control in Holland," by A. van Raalte, D.Sc., and J. Straub; "The Determination of Small Quantities of Methane," by H. R. Ambler, B.Sc., F.I.C.; and "The Fatty Acids and Component Glycerides of Indian Ghee," by R. Bhattacharya, Ph.D., A.I.C., and T. P. Hilditch, D.Sc., F.I.C.

### NORTH OF ENGLAND SECTION.

THE Sixth General Annual Meeting of the Section was held in Manchester on February 14th, 1931. The attendance was thirty-four, and the Chairman (Mr. G. D. Elsdon) presided.



A resolution of sympathy with Mrs. Miller and family on the death of Mr. James Miller was passed. The financial statement for the past year was adopted. The following officers and committee were appointed:

*Chairman*, C. J. H. Stock; ~~The~~ *Chairman*, J. Evans.

*Committee*, E. G. Jones, H. Haslam, H. Heap, A. Lees, A. R. Tankard, and J. Wood; *Honorary Auditors*, U. A. Coates and W. Marshall; *Hon. Secretary*, J. R. Stubbs.

The Chairman gave the Annual Address, and the following papers were read and discussed:—"The Rapid Quantitative Determination of Solid Saturated Fatty Acids," by Prof. T. P. Hilditch, F.I.C., and J. Priestman, Ph.D. (*Work done under the Analytical Investigation Scheme*); "A New Method for the Determination of Solid Unsaturated Fatty Acids," by L. V. Cocks, F.I.C., B. C. Christian, Ph.D., A.I.C., and G. Harding, F.I.C.

## Death.

WITH regret we record the death, on January 30th, of James Miller, a Member of the Society since 1922.

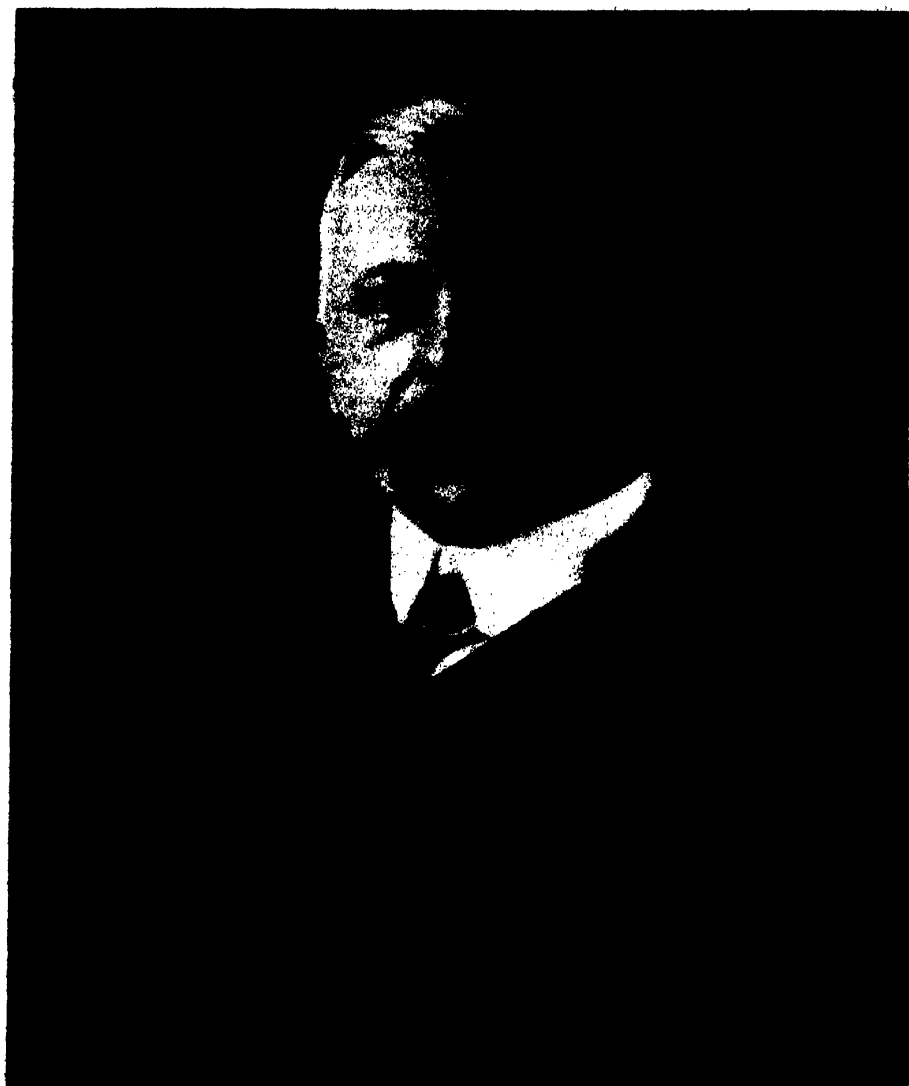
## Obituary.

### EDWARD WILLIAM VOELCKER.

THE passing away, on November 22nd, of our Past President, Edward William Voelcker, has made another gap in the too rapidly lessening roll of the survivors of the "Old Brigade" of those who were intimately associated with the work of our Society in its earlier, as well as in its later, days.

He was the third of five sons of the late Dr. Augustus Voelcker, F.R.S., and was born on July 14th, 1857, at Cirencester, during the time when his distinguished father occupied the chair of chemistry in the once celebrated Royal Agricultural College, which was then the only academical seat of agricultural training.

In 1864, the pressure of his duties as consulting chemist to the Royal Agricultural Society of England led Dr. Voelcker to resign his professorship at Cirencester and to migrate to London, where, as they severally emerged from the preparatory educational stage, he placed his sons, including the subject of this memoir, in the well-known school attached to University College, London. The eldest brother, George, proceeded to the College itself, having chosen a medical career which, unhappily, was closed by early death after a brilliant studentship. The second son, our other Past President, Dr. J. A. Voelcker, also proceeded to University College, and subsequently to Giessen; while "E. W." or "Will" (as



*E. W. Vacker*



his intimates affectionately and alternatively called him), having acquired an enthusiasm for chemistry under his then well-known chemical schoolmaster, Temple Orme, and having matriculated in the University of London, persuaded his father, in 1874, to allow him to go to the Royal School of Mines, where in due course in, 1877, he took his associateship, being awarded the De la Bêche Medal for proficiency in mining. His teachers there were Edward Frankland, Percy, Warrington Smyth and A. C. Ramsay, and among the best remembered of his college friends of those days were Arthur G. Phillips, George Seymour and Edgar Rathbone.

During the college vacations he used to frequent his father's laboratory in Salisbury Square, where he and the writer—who was then serving his pupilage—became from time to time fellow workers at the same bench, resulting in the foundation of a deep friendship which never waned during the fifty-five years that followed. After leaving the School of Mines he continued to work regularly in his father's laboratory, varying his experience as opportunities offered by visiting mines in various parts of England and Wales.

In 1879 he went on an exploratory trip on behalf of clients of his father to New Guinea, prospecting for phosphate deposits on various groups of islands adjacent to the north-east coast thereof, the chief group being that known as the Purdy Islands. He also spent some time in Australia, and after his return settled down as a permanent member of his father's staff. Shortly before his death, in 1884, Dr. Voelcker took the two sons into partnership, the elder brother, J. A. Voelcker, subsequently succeeding his father in the post of consulting chemist to the Royal Agricultural Society.

E. W. Voelcker joined our Society in 1889, and was elected a Member of Council in 1890. In 1894 he was chosen to succeed the late Charles Heaton in the office of Honorary Treasurer, and in this capacity his natural ability for finance became valuable to the Society, which not very long before had taken over the proprietorship of *THE ANALYST*, thus placing on the shoulders of the Treasurer more work and responsibility than devolved on his predecessor in earlier days, the duties of the office steadily increasing in exigency as the size and scope of our Journal developed. Voelcker continued to fill this office until 1910, when, after sixteen years of service, he was chosen to succeed our old friend, Mr. Tatlock (who it may be parenthetically remarked is still well and hearty in his ninety-fourth year) as President. Later on, from 1916 to 1920, during the absence on war service of his successor, Mr. Hinks, he again became our Acting Treasurer. In this connection it must be still further recorded that his willingness to place his ability for dealing with financial matters at the disposal of his professional brethren was not confined to the service rendered to our own Society, for he further earned our general gratitude by discharging, from 1918 to 1924, the still more onerous and complicated duties of the Honorary Treasurership of the Institute of Chemistry. On the Council of this latter body, in addition to his term of office as Treasurer, he served, in various periods, twenty-four years, including

two periods as Vice-President; while during five years he occupied the responsible office of Censor. During all this time, both in our own Society and in the Institute, he assiduously attended the constantly occurring meetings of the various committees on which so much of the spade work of such organisations necessarily devolves, and his conscientious attention to details, as well as the judgment of a well-balanced brain, will be long remembered by all those who had the satisfaction of serving with him. Apart, too, from his share in our corporate labours, many of us will have grateful personal recollection of wise advice always sympathetically given in cases of personal doubt or difficulty such as will occasionally arise in professional matters. His sense of right and wrong in matters of etiquette, as well as in matters of ethics, was always to be trusted.

His knowledge of general as well as of scientific matters was wide, and his mental ability such as might well have been devoted to original investigation; but he was modestly satisfied to leave in the hands of his brother the task of continuing the investigational work at Woburn and elsewhere entailed by successorship to the work of their father, restricting his chemical activity mainly to the everyday duties of the laboratory. That this involved no apathy or lack of grasp of the wider aspects of agricultural science was evidenced by the success with which he took over and carried on both the investigational work at Woburn and the multifarious advisory duties of consulting chemist to the Royal Agricultural Society during the time of Dr. Voelcker's absence in the service of the India Office on the tour which resulted in his memorable report of 1893, which formed the basis of the subsequent widespread development of Indian agricultural education. But it was with unconcealed relief that, on the return of Dr. Voelcker, he handed back to him the reins which he had temporarily held.

His first public appointments were those of Public Analyst for the Counties of Hereford and Northampton in 1894, and he subsequently became, with his brother, Joint Public Analyst for the Counties of Bucks. and Oxford and the Boroughs of Aylesbury and Banbury, being also Official Agricultural Analyst for Hereford and Northampton, and Deputy Agricultural Analyst for the Counties of Berks., Bucks., Northumberland, Oxford, the East Riding of Yorks., and the Isle of Ely. Some of these appointments he had recently resigned owing to failing health, being succeeded therein by his son and junior partner, Mr. Eric Voelcker, who, it is pleasant to realise, is carrying on the family chemical activity in the third generation.

"E. W." was much loved by those of his chemical brethren who were within the circle of his personal acquaintance, and it must be added that he was for very many years a popular member of the Savage Club. His favourite recreations were shooting and fishing, in both of which sports he was an adept.

He married in 1893 Jessie McCaskie, eldest daughter of Mr. R. Purvis Beattie, of Edinburgh, and leaves, including Mr. Eric Voelcker, three sons and one daughter.

BERNARD DYER.

## The Determination of the Milk Proteins.

By GEORGE M. MOIR, M.Sc., Ph.D., A.I.C.,

*Pedler Research Scholar of the Institute of Chemistry, 1928-1930.*

*(Read at the Meeting, November 5, 1930.)*

### III. PROPOSED MODIFIED METHOD FOR CASEIN.

ON the basis of a series of experiments which have been carried out, the following modification is proposed of the acetic acid method for the analytical separation of casein from milk:

Into a weighed covered beaker (100-150 ml.) pipette 10 ml. of the well mixed sample and weigh again quickly. Dilute the milk with about 50 ml. (*Note 1*) of distilled water which has been first warmed to 40-42° C. (*Note 2*). Add at once 1.5 ml. of 1.67 *N* (10 per cent.) acetic acid (*Note 3*), and then stir gently by rotating the stirring rod four times in the beaker (*Note 4*). After allowing the beaker to stand about 20 minutes, add 4.5 ml. of 0.25 *N* sodium acetate solution (*Note 5*), and, after stirring gently (*Note 4*), leave for at least an hour. Filter through a 9 cm. No. 42 Whatman filter, which it is desirable to fold in the fluted way to facilitate the operation. Wash the precipitate with distilled water three times by decantation and follow by two further washings in which the precipitate is broken up and transferred to the paper (*Note 6*). Finally, rinse the rim of the filter paper with a fine stream of water. The filtration and the washing should be carried out without interruption, and subsequently the casein adhering to the beaker and stirring rod should not be allowed to dry before being washed out with the sulphuric acid that is required for the Kjeldahl digestion. For this purpose about 20 ml. of water are placed in the beaker and about 5-7 ml. of the strong acid carefully poured down the side. The heat generated by gentle mixing helps to dissolve the casein, which is usually completely removed after three such treatments (*Note 7*). The filter paper and casein are added to the Kjeldahl flask into which the washings from the beaker have been poured; the usual quantity of sodium or potassium sulphate and a little copper sulphate are added before commencing to heat the digestion flask. It is desirable to evaporate the water with a small flame, as frothing is liable to occur just before the last of the water is boiled off. During digestion some of the acids from the fat condense upon the neck of the flask and may subsequently cause trouble by frothing during the distillation. These can be destroyed if the flask is allowed to cool when digestion is nearly complete, and about 50 ml. of water are carefully added and mixed with the contents. The fatty material is washed down by the condensation of steam during evaporation of the water. The determination is completed in the usual way and the ammonia collected in 40 ml. of *N*/10 sulphuric acid to which a few drops of sodium alizarine-sulphonate (1 per cent.) indicator has been added. The excess of acid is measured by titration with *N*/10 sodium hydroxide solution until the brown colour first develops a pink shade. The results

must be corrected for a blank properly carried out (in this case on a single filter paper and the same quantities of the other reagents), and expressed as per cent. of casein *nitrogen*. It is better to state the results in this way than as casein because of the existence of a slight doubt about the conversion factor.

NOTES.—1. The amount of water for dilution need not be exactly measured but an excess is more desirable than a deficiency.

2. The warm water facilitates the gathering of the casein into a clot, leaving a clear filtrate. The temperature must not exceed by more than one or two degrees that prescribed or there will be a risk of coagulating soluble protein.

3. The acetic acid must be added first, followed by the acetate, otherwise an opalescent filtrate results. The filtrate ought to be clear and filtration fairly rapid, and one advantage of the method is that if these requirements are not attained the results must be viewed with suspicion. The use of acetic acid alone will usually give a clear filtrate, but the results will be low by 1–2 per cent.

4. Excessive stirring will detach fat globules from the casein and will also leave some of the latter in a finely divided state, so that a cloudy filtrate results. Sometimes this can be remedied by passing the first 15 or 20 ml. twice through the filter paper, but this, on occasion, causes the remainder of the filtration and washing to be very slow, and the results unreliable.

5. The sodium acetate solution may be made by weighing out A.R. crystals, but too much should not be made at once, as moulds grow very readily in it. The amounts of the reagents are chosen to bring 10 ml. of the diluted milk to  $pH$  4.6, the isoelectric point of casein, and approximately this  $pH$  will be attained with most normal milks. Slight souring will not make much difference, but when the titratable acidity of 10 ml. of milk exceeds 2.5 ml. of  $N/9$  sodium hydroxide solution (0.25 per cent. of lactic acid) it may be desirable to investigate the  $pH$  of the filtrate (or, alternatively, add to the milk sufficient alkali to bring the titratable acidity back to 0.25 per cent.). It must be remembered, however, that owing to the possibility of proteolysis, casein determinations in sour or clotted milk are liable to be low. Palmer (*Missouri Agr. Exp. Sta. Research Bull.* 34, 1919) has shown that the best preservative of the milk proteins is formaldehyde (1.5–2 ml. of 40 per cent. formalin per litre will keep the sample at  $10^{\circ}C.$ ), but unfortunately this is liable to cause fat determinations to be slightly low.

6. I have made tests in which the casein precipitate was washed with an acetic acid and sodium acetate mixture of very low concentration, but of  $pH$  close to the isoelectric point of casein. The results scarcely differed from those obtained by washing the precipitate with water, so that it is doubtful whether the use of such a buffer solution should be recommended for this purpose.

7. Great care is necessary in this operation to avoid loss due to spurting of the acid during mixing, and also due to small pieces of casein which adhere to the stirring rod and the lip of the beaker. If the casein has become dry before rinsing out the beaker, its removal can be facilitated by moistening it with about 20 ml. of water containing 1 ml. of  $N/10$  sodium hydroxide solution and allowing

the beaker to stand for 5 to 10 minutes before adding the first portion of acid. The alkali solution must not be warmed before adding the acid, or ammonia may be lost.

This paper represents part of the work carried out during my tenure of the Pedler Research Scholarship of the Institute of Chemistry, the assistance of which and the interest of the members of the Pedler Fund Committee are gratefully acknowledged. I wish to add my appreciation of the facilities made available to me at the National Institute for Research in Dairying, and especially of the valuable advice of the chief chemist, Capt. J. Golding.

(Part IV, "The Determination of Albumin and Globulin," will be published in the April issue.)

## The Analysis and Composition of Vegetable Parchment used for Packing Dairy Products.

By PAUL ARUP, M.Sc., F.I.C.

*(Read at the Meeting, November 5, 1930.)*

THE following instructions relating to Vegetable Parchment to be used for the packing of butter were issued by the Department of Agriculture in Dublin under the Dairy Produce Act, 1924 (Regulations under Part II, Order No. 1, 1925):—

The vegetable parchment paper to be used for lining and wrapping shall be white in colour and free from any weighting material such as gums or sugars, etc. The texture shall be even and free from blotches, pin-holes, particles of metal and untreated material, and shall be such that, when torn, there shall be no appearance of fibres on the torn edges.

**WEIGHT.**—For lining bulk packages, the vegetable parchment paper should weigh not less than 25 lb. per ream of 480 sheets, measuring  $30 \times 20$  inches each.

For wrapping rolls, bricks and prints, the vegetable parchment paper should weigh not less than 18 lb. per ream of 480 sheets, measuring  $30 \times 20$  inches.

**PREPARATION OF VEGETABLE PARCHMENT PAPER.**—The parchment paper cut to the requisite sizes must, the night before it is to be used, be placed in a strong wooden tub or other approved vessel free from odour, containing a saturated boiling solution of salt brine with some excess salt in the bottom of the tub or other vessel and be allowed to remain in the brine overnight.

The parchment paper should not be crushed when placing it in the brine, but should be rolled up so that it will present a smooth appearance when taken out. The packages should be carefully lined with the parchment paper so as to avoid wrinkles and bare spaces.

At the suggestion of Mr. A. Poole Wilson, Chief Inspector of Dairying in the Irish Free State, a detailed investigation was made of the chemical and physical properties of a number of samples of vegetable parchment commonly in use for the packing of butter, with a view to obtaining data which might make it possible to lay down more definite standards for this commodity.

The results of this investigation are contained in the table on p. 152. The first 23 samples, numbered from 1 to 15 inclusive, were obtained from manufacturers or their agents under the description of "vegetable parchment." Where letters are used after the numbers, the samples composing each group were of the same material, differing only in weight, and the bracketed results were obtained from



samples of the respective groups. The 18 samples, numbered 16 to 33 inclusive, were received for analysis from various creameries in the Irish Free State. Nos. 34 and 35 are samples of grease-proof paper, or imitation vegetable parchment, included for comparison.

#### METHODS OF ANALYSIS.

**MOISTURE.**—One grm. of the sample, cut into strips not exceeding  $\frac{1}{4}$  inch wide, was weighed out in a stoppered weighing bottle (2" wide by 1" high) with straight side, and dried in a steam oven until constant in weight; the steam oven was fitted with air inlet and outlet, whereby there was a flow of ventilating air, preheated by passage through a tube in the body of the oven. Drying was completed within one hour.

**ASH.**—One grm. of the sample was incinerated in a platinum dish, precautions being taken to prevent loss of the very light ash through air currents. It was found advantageous to fold the parchment closely together before burning.

**FOREIGN MATTER.**—In many cases foreign matter would be detected by a careful inspection, and the presence of a number of substances is easily demonstrated by applying certain reagents to the surface of the paper. Thus *nitrogenous matter*, such as *glue*, *gelatin* or *casein*, is detected by applying Millon's reagent or a little powdered sucrose and concentrated sulphuric acid, the latter test giving a characteristic red colour in positive cases. In testing for *starch* by the application of iodine solution it should be noted that genuine vegetable parchments give a faint blue colour with iodine, owing to the presence of products produced by the action of the sulphuric acid used in the parchmentising process, on the cellulose. *Rosin* is tested for by boiling a few grms. of the paper with acetic anhydride and adding a few drops of concentrated sulphuric acid to the extract. *Ground-wood* is tested for by applying a 2 per cent. solution of phloroglucinol in a mixture of equal parts of concentrated hydrochloric acid and water to the paper. In the samples examined *free acid* was not present, and it was sufficient to test the aqueous extract of the sample with indicators such as methyl red and brom-phenol blue or methyl orange. *Sugars* are dealt with under a special heading below.

**WATER-SOLUBLE EXTRACT.**—To 5 grms. of the sample, taken from various sheets throughout the batch and cut into strips not more than  $\frac{1}{4}$  inch broad and  $2\frac{1}{4}$  inches long, were added 100 c.c. of distilled water in a 200 c.c. beaker flask which was kept on a steam bath for half an hour, the contents being stirred frequently. The water was then poured off through a filter into a tared basin in which it was evaporated to dryness on the steam bath. In the meantime the parchment strips were treated with a second portion of 100 c.c. of distilled water as before, and the process was carried on until four successive portions of 100 c.c. had been used for extraction and evaporated to dryness. A further 50 c.c. of water were used for washing out the filter and run into the basin so as to concentrate the extract near the centre of the basin, in order that solution in a small volume of water might be facilitated in the subsequent determination of reducing material.

When the residue had been dried as far as possible on the steam-bath it was further dried *in vacuo* until constant in weight; being slightly hygroscopic, it was

weighed as quickly as possible. In these investigations the dryings were carried out in a Mojonnier vacuum oven at 85–90° C. and 25 inches of vacuum; failing this, a vacuum desiccator could be employed.

**REDUCING MATERIAL CALCULATED AS DEXTROSE.**—The solid extract in the dish from the previous determination of extract was transferred to a 150 c.c. conical flask with 10 c.c. of hot water, used in three portions of 3 to 4 c.c. each, to allow of two rinsings. The reducing material was determined by iodimetric titration, as described by Hinton and Macara (ANALYST, 1924, 49, 2). In all cases except that of sample No. 19, 10 c.c. of 0.05 *N* iodine solution were used, with proportionate amounts of alkali and acid. On adding the 10 c.c. of iodine solution and 2.5 c.c. of 0.25 *N* sodium hydroxide, the temperature of the mixture was adjusted to 17.5° C. as quickly as possible, the flask was closed with a rubber stopper and placed in a water bath at 17.5° C. for 10 minutes, after which the contents were acidified with 2.5 c.c. of *N* sulphuric acid and titrated with 0.05 *N* thiosulphate solution in the usual way. A blank was carried out under the same conditions. For the calculation it was assumed that 1.410 grms. of iodine represented 1 gm. of glucose; the results must be regarded as empirical, and used in a comparative sense. The question of the composition of the water-soluble extract is dealt with under a separate heading below, where it is shown that in genuine vegetable parchment the reducing action can by no means be considered to be due to glucose alone.

**STRENGTH.**—The figures in the table represent the bursting strength of the samples in lbs. per square inch, as determined by the Ashcroft paper tester; the column headed "wet" contains results of tests made on the samples immediately after immersion in water at 80° C. for 15 minutes, and that headed "dry" contains the results of tests on the corresponding samples before wetting. The column headed "Ratio per cent." shows the strength wet calculated as the percentage of the strength dry. In all cases the results represent the average of ten determinations on pieces cut from different sheets or parts of sheets.

**WEIGHT.**—This was determined by means of the "Pocket Gram Scale" (made by L. Schopper of Leipzig), which gives readings in grms. per square metre. As the figures represent the average of ten determinations in each case, the use of a direct reading scale saves a considerable amount of labour. A convenient scale for conversion of results into lbs. per ream of 480 sheets, measuring 20 × 30 inches, is made by R. Stewart & Co., of Glasgow.

**SUPPORT OF MOULD GROWTH.**—Experiments were made to ascertain how far the parchment would support the growth of mould under favourable conditions as regards moisture and temperature. Circles of the parchment, about 4" in diameter, were placed in sterile 4½" Petri dishes, and moistened with water containing a suspension of spores of *Penicillium* and *Cladosporium* species, these being among the most active moulds which produce rancidity. The dishes were incubated at 25° C. and examined from time to time, water being added when necessary, to keep the parchment moist but not flooded. A final judgment could be made after 14 days.

## RESULTS OF ANALYSES.

The quantitative results are summarised in the accompanying table:

TABLE I.

No.	Description.	Water. Per Cent.	Ash. Per Cent.	Extract. Per Cent.	Reducing matter calcu- lated as	Reducing matter in	Strength in lb. per sq. inch.			Weight per ream. lbs.
					dextrose.	extract.	Wet.	Dry.	Ratio. Per Cent.	
1	Vegetable parchment	8.45	0.28	0.78	0.13	17.0	13	33	39	29
2	do.	—	—	0.78	0.14	18.0	8	22	36	18
3	do.	9.05	0.27	1.08	0.21	19.4	7	19	37	23
4A	Vegetable parchment First grade Pure rag	—	—	0.78	0.12	15.4	12	26	46	21
4B		—	—				14	28	50	25
4C		—	—				18	29	62	27
5A	Vegetable parchment Second grade	—	—	1.14	0.12	9.5	12	25	48	22
5B		—	—				12	23	52	25
6	Vegetable parchment	9.10	0.37	0.65	0.09	11.3	12	27	44	28
7A	"Real vegetable parchment"	8.32	0.29	0.66	0.09	13.7	7	20	35	19
7B							6	18	33	18
7C							9	21	43	21
7D							10	24	42	24
7E							15	30	50	29
7F							11	28	40	29
8	Vegetable parchment	—	—	0.83	0.08	9.6	9	28	32	25
9	do.	9.38	0.40	1.30	0.22	17.0	20	34	59	30
10	do.	8.75	0.35	1.42	0.17	12.1	15	24	62	23
11	do.	9.13	0.59	1.67	0.21	15.1	18	32	56	29
12	do.	8.75	0.34	0.70	0.16	22.9	10	19	53	19
13	do.	8.15	0.34	1.16	0.25	21.5	14	25	56	26
14	do.	8.60	0.28	1.14	0.21	18.4	9	16	50	16
15	do.	9.15	0.33	1.02	0.17	17.0	16	32	50	29
16	do.	9.16	0.25	0.86	0.12	14.0	13	25	52	28
17	do.	9.27	0.38	0.78	0.14	18.0	7	22	32	25
18	do.	9.08	0.26	0.85	0.14	16.5	16	29	55	30
19	do.	10.75	0.12	7.01	0.75	12.0	13	28	46	31
20	do.	—	—	0.60	0.13	21.7	15	32	47	29
21	do.	—	—	0.78	0.19	24.4	15	29	52	31
22	do.	—	—	0.70	0.11	15.7	10	29	34	31
23	do.	—	—	0.80	0.11	13.8	11	31	35	29
24	do.	9.69	0.29	0.75	0.12	16.0	19	31	61	27
25	do.	8.45	0.32	0.96	0.11	11.5	18	31	58	30
26	do.	8.95	0.40	1.05	0.13	12.4	14	30	47	30
27	do.	10.10	0.26	1.12	0.21	18.8	17	34	50	31
28	do.	11.70	0.26	0.84	0.20	23.8	22	35	63	30
29	do.	10.08	0.41	1.18	0.14	11.9	15	28	54	29
30	do.	9.06	0.40	1.30	0.12	9.2	17	29	59	29
31	do.	8.16	0.39	1.24	0.22	17.8	9	25	36	26
32	do.	8.97	0.40	1.18	0.20	17.0	16	30	53	28
33	do.	—	—	0.98	0.13	13.3	13	29	44	28
34	Greaseproof bleached	8.72	2.67	0.76	0.26	34.2	nil	29	nil	18
35	Greaseproof unbleached	8.37	0.93	0.47	0.17	36.2	nil	21	nil	18

The qualitative tests for *foreign materials* gave negative results in all cases. The exceptional sample, No. 19, gave a somewhat stronger colour with iodine than usual, but not sufficiently strong to indicate the presence of added starch.

**SUPPORT OF MOULD GROWTH.**—All the samples except No. 19, when tested in the manner described, showed a somewhat attenuated growth of mould. Sample No. 19, which contained far more carbohydrate material than the other samples, showed a vigorous growth.

The following table and account give particulars of simultaneous comparative tests for support of mould growth with six samples of parchment having different percentages of soluble extract:

Sample No. . . . .	22	24	16	10	11	19
Extract, per cent. . . . .	0.70	0.75	0.86	1.42	1.67	7.10

The samples were examined from time to time over a period of 14 days, using a low-power microscope where necessary. Sample No. 19 developed a visible mould growth in the course of 4–5 days, and long before the rest of the samples. Nos. 10 and 11 had developed less vigorous growths than No. 19 at the end of the 14 days. Nos. 22, 24 and 16 supported growth more sparingly than the rest, and microscopic examination especially showed that the mould hyphae were not nearly so plentiful as in Nos. 10 and 11. These experiments, therefore, show a definite connection between extract content and power to support mould growth.

It should, however, be emphasised that if a parchment has become heavily infected with mould spores through storage in an unsuitable place, it may easily develop mould spots under practical conditions of use, even if of satisfactory chemical composition. In such cases the parchment must be very thoroughly sterilised in boiling brine, because the mere presence of salt, even in high concentration, is not sufficient to check the growth of some of the common moulds, notably the *Cladosporium* species.

#### DISCUSSION OF RESULTS.

Before discussing the above results in detail it may be of use to tabulate some previously published results in the following condensed form:

TABLE II.

Observers.	No. of samples.	Description of samples.	Water. Per Cent.	Ash. Per Cent.	Water-soluble extract. Per Cent.	Sugar. Per Cent.
Burr and Wolff <sup>1</sup>	26	Commercial parchments	7.13 to 10.31	0.34 to 17.10		0 to 25.78
Burr, Wolff and Berberich <sup>2</sup>	58	do.	5.49 to 13.10	0.23 to 17.10	0.05 to 31.10	
Weiss <sup>2</sup>	40	Parchment and parchment substitutes	5.91 to 11.32	0.32 to 10.32	0.38 to 33.9	

<sup>1</sup> ANALYST, 1910, 35, 135.

<sup>2</sup> ANALYST, 1912, 37, 465.

<sup>2</sup> Z. Untersuch. Nahr. Genussm., 1923, 46, 301.

On the basis of the results summarised in Table II, the following maximum standards were suggested by Burr, Wolff and Berberich, and adopted by the German Dairy Association:—Ash, 3; water-soluble extract, 8; sugar, 8 per cent.

It will be seen that the figures obtained in the present investigation point to a considerably higher standard of purity.

**MOISTURE.**—This varied from 8.15 to 11.70 per cent. As regards the four samples over 10 per cent., No. 19 was the exceptional sample, containing an excessive amount of water-soluble extract, whilst Nos. 27, 28 and 29 represented batches which were found to have been stored under unsuitable conditions. It is suggested that 10.0 per cent. would be a suitable maximum standard. Excessive moisture, such as would be occasioned by the presence of undue amounts of sugar or glycerin, would favour the growth of moulds. In general, it may be said that good vegetable parchments contain between 8.0 and 10 per cent. of moisture. An unduly low moisture content would probably be accompanied by a lack of pliability.

**ASH.**—The figures for vegetable parchment varied from 0.12 to 0.59 per cent., those for the greaseproof papers, especially the bleached sample, being considerably higher. As a maximum standard, the figure 0.45 per cent. may be tentatively suggested. The maximum value of 3.0 per cent., adopted in Germany, is intended to allow for the impregnation of the parchment with salt. It is claimed that this practice prevents mould growth on the parchment; and while this is no doubt quite true, it is considered better, from several points of view, to purchase a pure quality of parchment and to steep this in hot brine before use, as recommended in the instructions quoted at the beginning of the account of the present investigation. In this way the advantage of the sterilising effect of the hot brine is secured, while the procedure is to be recommended as being, on the whole, more systematic and amenable to control.

**WATER-SOLUBLE EXTRACT.**—For vegetable parchment, the figures vary from 0.65 to 7.01 per cent. If sample No. 19 is omitted, the variation is from 0.65 to 1.67 per cent. These figures show, on the whole, far less variation than those quoted in Table II, and it is obvious that the parchments under examination were far better in quality than the majority of those analysed by previous observers. As the water-soluble extract is capable of supporting mould growth, it will be argued that the paper should be washed free from this matter in the process of manufacture, but it is stated by the makers that such a course would result in lack of pliability in the product. It is, no doubt, the hygroscopic nature of the extract which enables it to impart suppleness to the parchment; the same reason underlies the occasional use of glycerin for this purpose.

An unduly high percentage of extract will cause the parchment to have a brownish tinge, as was seen in the case of sample No. 19, which contained 7.01 per cent. of extract. As the proportion of reducing sugar to the total extract in this sample came within the usual limits, it may be presumed that the excessive amount of extract was due, not to added sugar, but to products of the action of sulphuric

acid on cellulose formed in the parchmentising process and not removed by washing. In this case the aqueous extract was neutral, as usual. From the results quoted under "Support of Mould Growth" (p. 153), it is seen to be important that the water-soluble extract should be kept within moderate limits; and, judging from the general trend of the results, a maximum of 1.20 per cent. is suggested for this constituent.

REDUCING MATERIAL, CALCULATED AS DEXTROSE.—Excluding sample No. 19, the figures for vegetable parchment varied from 0.09 to 0.25 per cent. The percentage of reducing material in total extract varied from 9.2 to 24.4 per cent. It is suggested that these figures may be used as a basis for determining the presence of added glucose, 1 per cent. of which would raise the percentage to somewhere about 50; in most cases this figure would be exceeded.

From the results of the examination of the composition of the water-soluble extract, it is evident that only a relatively small amount of the reducing material in genuine vegetable parchment consists of dextrose; the major portion appears to be lignone or its decomposition products. The calculation to dextrose is, therefore, only made in order to have a standard for purposes of comparison, and because the object of the determination is largely to detect added glucose.

BURSTING STRENGTH.—As the Regulations quoted at the beginning of this paper prescribe 25 lbs. per ream as the minimum weight for the purpose of lining bulk packages, the bursting strength of the samples of this weight and over may be considered separately. The figures for this group vary from 22 to 35 lbs. per square inch; for 25 to 26 lbs. per ream the strength varied from 22 to 28 lbs. per square inch, and for 30 to 31 lbs. per ream from 29 to 35 lbs. per square inch. As regards the minimum value of 22 lbs. per square inch observed for sample No. 17, it will be noted that this is the only sample showing the strength, wet, to be under 33 per cent. of the strength, dry (*vide infra*), while sample No. 5B, which showed the comparatively low value of 23 lbs. per square inch, was supplied under the description of second grade. It is, therefore, suggested that 25 lbs. per square inch might fairly be adopted as a minimum for this class of parchment.

In the Regulations quoted at the beginning of this paper, the minimum weight of parchment for wrapping rolls, prints, bricks, etc., is 18 lbs. per ream. The strengths of samples from 18 to 24 lbs. per ream varied from 18 to 24 lbs. per square inch. For this class of paper it is suggested that 18 lbs. per square inch might be taken as a minimum.

The ratio of the strengths in the wet and dry states affords the most reliable criterion for genuine vegetable parchment, especially as it has a direct connection with practical conditions. It may be regarded as covering other tests, such as that of boiling with 10 per cent. sodium hydroxide solution for 15 minutes, after which the parchment should not have disintegrated, or that of observing the behaviour of the parchment on scorching with a small flame.

It will be noticed that the greaseproof papers break down entirely in the bursting-strength test after having been wetted.

The rule that the strength, wet, should be at least 33 per cent. of that dry, may safely be taken as a standard for genuine vegetable parchment, and it will be seen that all of the samples from 1 to 33 inclusive (41 in all), except two (Nos. 8 and 17), conform to this requirement, the two exceptions falling short by 1 per cent. It is understood that all weight and strength test results are averages of at least ten separate trials.

#### COMPOSITION OF WATER-SOLUBLE EXTRACT.

It was considered desirable to examine the general nature of the substances determined and examined in routine analyses as water-soluble extract in genuine vegetable parchment. Several quantities, each of 1 kilo., of sample No. 32, were extracted with hot distilled water on the steam-bath, the aqueous extract being concentrated by evaporation over steam. The extraction was not made quantitatively, as this would have involved the evaporation of inconveniently large amounts of water. The extract from 1 kilo. of parchment was concentrated to 100 c.c., treated with charcoal and filtered. To the filtrate were added 400 c.c. of 96 per cent. alcohol, which caused the precipitation of a white substance resembling dextrin; this was well washed with 90 per cent. spirit, filtered off and dried. One kilo. of parchment yielded 1.7 grms. of this substance.

The alcoholic filtrate was evaporated to dryness, and the residue was extracted with 95 per cent. alcohol; the alcoholic solution, on evaporation to dryness, yielded a brown solid, 1 grm. being obtained from 1 kilo. of parchment.

(A) EXAMINATION OF SUBSTANCE PRECIPITATED BY ALCOHOL.—This was a white amorphous substance with a slight greyish tinge, resembling dextrin, giving gummy solutions in water, which produced a purple coloration on the cautious addition of iodine. When the powder was stained with iodine solution, and viewed under the microscope, it showed two distinct localised colorations, *viz.* deep reddish brown and light purple. Staining with ferric ferricyanide and with aniline acetate solutions in a similar manner showed distinctly localised blue and red colorations, respectively. These indications point to the substance being a mixture of lignone and cellulose decomposition products, which supposition was borne out by the analytical data given below.

The solution gave a red coloration when warmed with phloroglucinol in hydrochloric acid solution; it reduced Fehling's solution, and in cold solution decolorised permanganate slowly. A 2 per cent. solution in water was laevo-rotatory, showing a specific rotation of  $[\alpha]_D = -46.6^\circ$  (calculated on the substance, less ash). On heating this 2 per cent. solution with dilute hydrochloric acid on the steam bath it became dextro-rotatory, a constant value being obtained after one hour's heating with 10 per cent. of the concentrated acid or after 2 hours with 2 per cent. of acid. The figure attained was  $[\alpha]_D = +25.8^\circ$  (calculated on the ash-free substance). This would correspond with the specific rotation of a mixture of dextrose and xylose, approximately in the proportion of one to four, assuming these two sugars to be the product of hydrolysis. The laevo-rotatory power of the original

substance may be assumed to be due to the predominance of a pentose-yielding complex.

On treatment of the hydrolysed material with phenylhydrazine in the usual way, a dark brown product was obtained, which after four crystallisations from alcohol yielded a small amount of crystalline material (m.pt.  $206^{\circ}\text{C.}$ ), which was identified as glucosazone; this may represent either or all of the three sugars—dextrose, mannose or laevulose, as these have all been obtained from wood by various observers. (See "Power Alcohol," by Monier-Williams.)

The material gave the following results on analysis:—Ash, 18.47 (chiefly calcium sulphate with a little ferric oxide); sulphur, 3.56; pentosan (by Kröber's method), 58.1 per cent. (calculated on the material, less ash); methoxyl, nil; acetic acid (by distillation with dilute sulphuric acid), nil.

With regard to the sulphur content of 3.56 per cent., this was represented by 0.15 per cent. of sulphur in the original parchment. It was thought possible that carbohydrate sulphuric esters, such as described by Cross and Bevan ("Cellulose," 1918, p. 50), might be present, but this was negatived by the fact that aqueous extracts of parchments invariably remained neutral during evaporation ( $\text{pH } 4.6\text{--}5.2$ ), while the material under consideration similarly remained neutral on boiling for 6 hours in 2 per cent. solution in water. The sulphur is, therefore, present as calcium sulphate.

The figures given under pentosan are based on the production of furfuraldehyde on distillation with 12 per cent. hydrochloric acid under the standard conditions of the Kröber method. The reducing properties of the original substance are to be expected from the known action of sulphuric acid on cellulose. (See Cross and Bevan, "Cellulose," 1918, p. 53.)

From the above observation it may be concluded that this material consists essentially of carbohydrate material, being a mixture of pentose- and hexose-yielding complexes, the former predominating. The lignone material which yields acetic acid and reduces permanganate instantaneously (see the following section) is absent.

(B) EXAMINATION OF SUBSTANCE SOLUBLE IN ALCOHOL.—This was a brown hygroscopic powder; it reduced Fehling's solution readily and also decolorised permanganate instantaneously in cold solution. On treatment with phenylhydrazine in the usual way, it behaved similarly to the material described under (A), the crude product yielding, after four crystallisations from alcohol, a small amount of glucosazone (m.pt.  $206^{\circ}\text{C.}$ ). Polarimetric examination showed  $[\alpha]_D = -22.7^{\circ}$  (calculated on the substance, less ash).

The following results were obtained on analysis:—Ash, 11.53; pentoses (by Kröber's method), 6.25; methoxyl, nil; and acetic acid (on distillation with dilute sulphuric acid), 31.30 per cent. (Pentoses and acetic acid were calculated on the substance, less ash.)

The determination of acetic acid was carried out as follows:—One grm. of the material was dissolved in 100 c.c. of recently boiled water and 40 c.c. of *N*



sulphuric acid; a little pumice was added, and the mixture was distilled in a Polenske apparatus. One hundred c.c. of distillate were collected and titrated with *N*/10 sodium hydroxide solution (phenolphthalein as indicator). Successive quantities of 100 c.c. of water were added to the mixture in the flask and distilled off until a constant blank value was obtained on titration. The sum of the titrations, with allowances for blanks, gave the total yield of acetic acid. The acid was identified as practically pure acetic acid (containing traces of formic acid) by the usual reactions and by an electrometric determination of the *pH* value of a solution of known titre obtained after evaporating the neutralised distillates to dryness, dissolving in water, acidifying and distilling as before.

The unsaturated nature of the material and its yield of acetic acid on hydrolysis are characteristic of lignone. (See "Researches on Cellulose," by Cross and Dorée.)

The methoxyl which might be expected to be present in this material has evidently been completely split off by the action of the strong sulphuric acid used in the parchmentsing process. (Cf. Hägglund, *Chem. Ztg.*, 1919, 90, 186.)

The comparatively small amount of furfuraldehyde obtained by the Kröber distillation process has been provisionally calculated as pentose. It may, however, be due to the presence of some of the material described under (A), which is also indicated by the laevorotation of the material and the fact that, when exhaustively extracted with alcohol, the rotation of the alcohol-soluble portion is found to be practically nil.

The examination of the water-soluble extract under headings (A) and (B) shows that it consists of decomposition products of lignocellulose. The analytical methods which have been adapted, mainly from the investigations of Cross and Bevan, Cross and Dorée, and Klason, may perhaps be found useful for purposes of analytical control. It may be pointed out that the reducing material determined by iodine titration in genuine vegetable parchment probably represents substances falling under at least three different headings—(1) Simple sugars; (2) complex carbohydrates; (3) unsaturated lignone material, which reduces permanganate far more readily than the sugars.

**SUMMARY.**—1. The following determinations were carried out on 41 samples of vegetable parchment and 2 samples of greaseproof paper:—Water, per cent.; ash, per cent.; water-soluble extract, per cent.; reducing material (as dextrose), per cent.; bursting strength wet and dry, and weight, in lbs. per ream. The samples were also examined as to their power to support mould growth. The methods adopted are described and the results are tabulated.

2. As the result of these determinations, the following additional standards are tentatively suggested:—Water, maximum 10.0 per cent.; ash, 0.45 per cent.; water-soluble extract, 1.30 per cent.; bursting strength (by Ashcroft tester), minimum 25 lbs. per square inch for parchments of 25 lbs. per ream, and 18 lbs. per sq. in. for 18 lbs. per ream. The strength immediately after wetting, as described, to be at least 33 per cent. of the strength of the unwetted sample.

3. The percentages of reducing material, as dextrose, in the water-soluble extract have been calculated as a guide to the detection of added sugar.

4. Certain standards adopted in Germany (*viz.* 8 per cent. of extract and 3 per cent. of ash), are considered far too high, being based on additions of sugar and salt, respectively. An unduly high content of extract renders the parchment particularly susceptible to attack by mould.

5. The water-soluble extract from genuine vegetable parchment was examined, and found to consist of decomposition products of lignocellulose. Methods by which they may be characterised have been adapted chiefly from the investigations of Cross and Bevan.

I wish to thank the Department of Agriculture, Irish Free State, for permission to publish this paper.

DEPARTMENT OF AGRICULTURE,  
BUTTER TESTING STATION, DUBLIN.

#### DISCUSSION.

The PRESIDENT remarked that he was sure the very fact that so little literature could be found on this subject would make these results of great value. He then welcomed to the meeting Mr. Faber, representing the Danish Government, and an old member of the Society.

Mr. HARALD FABER, after thanking the Society for the kind invitation to be present to hear this very interesting paper, said that he had great pleasure in proposing a vote of thanks to Mr. Arup. He was really surprised at the number of interesting details which had been extracted from such a subject. The chief interest which, he believed, Mr. Arup had in mind was to find out what composition parchment paper for wrapping butter should have, so as to avoid the growth of mould upon it. That side of the question had been studied in Denmark to some extent, but not so thoroughly as in the work under discussion.

When he received the invitation to be present at this meeting, he (Mr. Faber) had written to the Chief Inspector in Copenhagen, Mr. T. Lohse, who had to deal with this subject, and who had kindly sent a few notes from which Mr. Faber abstracted the following:

When the Chief Inspector took over his duties he found parchment paper being used which sometimes contained as much as 25 per cent. of sugar. (Mr. Arup had found a similar kind of paper used in Germany.) This was very bad for use in connection with butter, as it induced mould to grow. He had also found paper containing as much as 10 per cent. of glycerin. (Mr. Faber here mentioned that in Denmark they issued a parchment paper with their National Brand for butter wrapping, and the Chief Inspector had control of the specifications for this paper.) He had, therefore, been able to stipulate that the paper must be free from sugar or dextrose, but he had at first allowed 3 per cent. of glycerin, because he had found that paper was easier to handle if it contained a certain amount of glycerin, and 3 per cent. did not seem to encourage the moulds. Later, however, he eliminated that also, and tried paper entirely free from glycerin, and no objection was taken to its use. Various papers were tested by wrapping them round butter and infecting them with spores, and it was found that paper free from glycerin or sugar was very resistant to mould growth. Mr. Lohse had had one peculiar experience with a particular kind of paper free

from sugar and glycerin, which was very apt to grow mould; he had come to the conclusion that the paper during manufacture, or very soon after, must have been infected with moulds, and when steeped in boiling water for some time and dried no mildew grew on it. It had recently been a practice, when packing margarine in parchment paper, to have it lined on the inside with a thin layer of paraffin wax. This would make it easier for the packer to handle, but it was more expensive, and offered no advantage, so far as resistance to mould growth was concerned; therefore, its use was not likely to spread. Most of the parchment paper used at present in the Danish dairies was derived from Belgian sources (as also, he believed, was that used in Ireland), but they had recently had an English paper from a mill in Kent which, on analysis, was found to be entirely free from sugars and glycerin, and was very good paper. They were trying to find other mills in this country where they could buy good parchment paper, as, naturally, when sending butter to England, they would like to be able to wrap it in English paper.

Mr. McLACHLAN asked whether Mr. Arup had made any attempt to determine metallic impurities in the papers.

Mr. W. PARTRIDGE said that he would only touch on the side issues of this paper. This was the second time recently that he had heard of attenuated moulds; the previous occasion was when G. Smith, working on the mycology of textile fabrics, was getting them in the mildew on yarns and cotton cloths. In Mr. Arup's selection of moulds to determine the readiness of mould growth, he had noticed that *Penicillium* and *Cladosporium* had been taken. He supposed that this was because they were both coloured and therefore easy to see. *Cladosporium* was a cold-store mould which grew on meat—did it grow on butter? (This was answered in the affirmative.) He remarked that had he been asked to choose a mould for this purpose he would have suggested *Fusarium*. He quite appreciated that Mr. Arup would prefer a coloured mould, which would show up on the white paper, otherwise *Oidium lactis* would seem to be the most suitable in this connection. He would also like to make a point regarding the suggestion that paper should be dipped in hot brine. Had Mr. Arup given any strength for this brine? His reason for asking this was that in the case of brine for pig's flesh it was supposed to contain 23–28 per cent. NaCl, but often by the time it was sold as bacon it had been through brine containing only 12–13 per cent. NaCl and a few million bacteria per c.c. If the Irish Government were going to stipulate for immersion in brine, they ought to state definitely that the brine should be discarded before the sodium chloride percentage fell below a certain number. Finally he remarked on the use of the word "dextrose," and queried whether the word "glucose" should have been used.

Mr. A. L. BACHARACH called attention to a practical problem, arising especially over papers used for wrapping small packets intended for the retail market, namely, resistance to creasing. The several methods devised for measuring tensile strength did not give any information about the extent to which one could go in creasing or crushing a paper without breaking it. A method for rapidly measuring this, even approximately, would be very useful. Like the Danish authorities, he had found parchment paper from a Kent mill (presumably the same) quite satisfactory, though occasionally a sample exceeded the very low limit set for free acidity.

Mr. ARUP, replying, thanked Mr. Faber for his very helpful remarks. It appeared that Mr. Faber's friend had been working along similar lines to those he himself had followed, and he hoped they would be able to get in touch with each other on the subject. He had not come across any glycerin; he would like to

know the method used for determining this. In connection with paper he queried whether, when speaking of freedom from sugar, one meant actually chemically free from all traces of sugar or not. According to statements by manufacturers, if the carbohydrate were completely washed out, the paper would be too brittle. He had chosen *Penicillium* and *Cladosporium* because they were the most common moulds. *Oidium* was colourless. With regard to the brine, one should use dry salt of reasonably pure quality; spores gathered on the surface of damp salt. He had included in his paper directions for making the brine. Regarding the reaction, all soluble extracts were neutral, ranging between methyl orange and methyl red. The word "dextrose" was used for the pure chemical compound, whereas "glucose" was applied to the commercial product. In reply to Mr. Bacharach, Mr. Arup said that attempts to avoid this had been made by specifying that the paper must be of a certain weight. There was not nearly so much creasing if pure vegetable parchment were used as was the case with the imitation grease-proof paper. He had never found any metallic impurities, such as might have been used for weighting the paper.

Replying to Mr. Bolton, he said that he was rather loath to lay down definite standards (though he had put forward suggestions), as there was so often a tendency to regard them as the ultimate perfection. Very little technical information could be obtained from the manufacturers, and one had to decide what would be a fair standard from the results at one's disposal.

## The Fatty Acids and Component Glycerides of Indian Ghee.

By R. BHATTACHARYA, Ph.D., A.I.C., AND T. P. HILDITCH, D.Sc., F.I.C.

(Read at the Meeting, February 4, 1931.)

OWING to climatic and other factors it is difficult to preserve butter as such in good condition in India, and, moreover, it is not suited to Indian methods of cooking or sweetmeat making. Butter or cream is, therefore, almost universally clarified before sale, and is then known as ghee; when properly clarified, it has a pleasant odour and keeps indefinitely if stored in porous earthenware vessels, although rancidity develops in course of time when ghee is kept in glazed vessels or tins. Ghee is usually prepared from the milk of the cow or the buffalo; goat's milk, although considerably used in the Northern provinces, is not often churned for butter.

Ghee of the highest quality, clear white or yellow in colour and with a perfectly fresh butter-like flavour, is the subject of the present communication. This is generally prepared by collecting the thick layer of "skin" which forms when

boiled milk is left at a moderate warmth, and mashing and churning the accumulated layers, cold water being added towards the end as the fat rises. The buttermilk or whey is separated from the fat, which is very gently heated in an open pan, strained and stored.

The second quality ghee, which appears to be the pattern for most "ghee substitutes" imported into India, is underclarified, and in consequence soon develops its characteristic sharp rancid taste and peculiar flavour. It results either from the churning of fresh milk in open pitchers by means of a bamboo staff forked at the lower end and rotated by a twisted rope (a method dating back to the earliest Vedic period), or by curdling the milk with curd or lactic acid bacilli and then churning the curdled milk.

Two samples of buffalo and of cow ghee (first quality) have been investigated. In the first instance the fats had been prepared at a temperature not exceeding 40° C. from the milk of animals fed on a ration of dry grass and green millet with concentrates containing cotton-seed, sesamé-cake and lentil husks (Tur-chuni); the buffaloes were of Murrah breed, and the cows were three quarter Indian and one-quarter Ayrshire crossbred. These specimens are referred to as buffalo No. 1 and cow No. 1, and were, respectively, clear white and very pale cream in colour.

As the quantity of material was not sufficient for examination of the glyceride structure, further supplies were obtained, but these consisted of market samples from pasture-fed animals; the pasture-fed buffalo ghee (No. 2) was almost white, whilst the pasture-fed cow ghee (No. 2) was of a full yellow colour, and the odour of both was, again, perfectly fresh and free from rancidity. All the samples were kindly obtained for us by the Director of Industries, Central Provinces, Nagpur.

The general analytical characteristics of the ghees were as follows:

	Buffalo.		Cow.	
	No. 1.	No. 2.	No. 1.	No. 2.
Sap. equivalent . . . . .	252.3	251.0	252.0	249.2
Iodine value . . . . .	32.5	33.5	35.2	36.0
Reichert-Meissl value . . . . .	28.0	30.9	25.2	26.0
Polenske value . . . . .	1.4	2.2	1.4	1.9
Kirschner value . . . . .	24.6	25.6	20.9	20.6
Refractive index, $n_D^{20}$ . . . . .	1.4467	1.4462	1.4475	1.4470

COMPOSITION OF THE FATTY ACIDS OF THE FOUR SPECIMENS OF GHEE.—The method employed was in all essentials identical with that which was described fully in the ANALYST by one of us and E. E. Jones (1929, 54, 75). The total mixed fatty acids from 250–500 grms. of fat were carefully distilled in steam for four or five hours, during which time about 3 litres of aqueous condensate were collected. The "steam-volatile acids" were worked up, as described in the earlier paper, but it has been found that very small quantities of unsaturated acid are

present in the steam-distillate and are left in the residue from the fractional distillation of the volatile acids. The iodine value of this residue is, therefore, determined, and the unsaturated acid calculated as oleic acid; the amount is too small appreciably to affect the accuracy of the oleic acid figure, but its recognition permits a more correct value for the mean equivalent of the saturated acids (usually caprylic and capric) in this particular residue (usually only 1.5–2.5 grms.) to be ascertained. The acids non-volatile in steam are resolved by the lead-salt-alcohol procedure into groups of "solid" and "liquid" acids, which are converted into the respective esters and fractionally distilled in the manner previously described.

A typical set of the complete experimental data is given for one fat; but, as these figures occupy considerable space, the analytical results as a whole are summarised in Table II, wherein will be found the calculated increments of each acid present in the "steam-volatile," "solid" and "liquid" groups of fatty acids.

Precise identification of the majority of the commonly-recognised butter acids was thought unnecessary, but the acids present in the residual fraction of the "solid" esters from buffalo ghee No. 1 were submitted to fractional crystallisation from ethyl acetate and alcohol several times, when a specimen which melted at 74° C. was finally obtained, thus affording definite evidence of the presence of arachidic acid, or, at least, of an acid of higher molecular weight than stearic acid.

TABLE I.

*Typical Experimental Data (Buffalo Ghee No. 2).*

The ghee (250 grms.) yielded 14.1 grms. of steam-volatile and 216.5 grms. of non-steam-volatile acids; the latter were resolved by lead salt separation into "solid" acids (56.7 per cent., iodine value 7.2) and "liquid" acids (43.3 per cent., iodine value 68.8).

*"STEAM-VOLATILE" ACIDS.*

No.	Grms.	B.pt. °C.	Pressure.	Mean equiv- alent.	Acids.				
					Buty- ric. Grms.	Cap- roic. Grms.	Cap- rylic. Grms.	Cap- ric. Grms.	Oleic. Grms.
	In aqueous solution	..	..	..	1.65				
	In recovered ether	..	..	..	0.14				
1	14.0	35/ 85	Atmospheric		0.07				
2	7.3	85/ 90			0.43				
3	0.58	90/157			0.30				
4	1.32	157/161			1.23				
5	2.31	161/165		92.1	1.89	0.42			
6	2.84	165/170		94.9	1.98	0.86			
		170		95.6	0.47	0.23			
8	2.86	119/140	Reduced	102.0	1.23	1.63			
9	1.54	Residue (iod. val. 14.4)		162.0			1.20	0.09	0.25
Totals:					9.39	3.14	1.20	0.09	0.25

## METHYL ESTERS OF "SOLID" ACIDS S.

Primary fractionation.					Refractionation.				
No.	Grms.	B.pt./ 1 mm. °C.	Saponi- fication equiva- lent.	Iodine value.	No.	Grms.	B.pt./ 1 mm. °C.	Saponi- fication equiva- lent.	Iodine value.
S1	29.62	40-144	258.7	2.1	S11	0.84	81-100	226.2	nil
					S12	1.48	100-109	239.4	1.0
					S13	2.28	109-118	245.5	0.9
					S14	2.76	118-128	254.0	1.0
					S15	5.09	128-129	255.6	0.8
					S16	6.31	129-130	262.8	0.8
					S17	4.63	130	271.7	2.9
					S18	3.87	Residue	279.1	6.9
						27.26			
S2	11.79	144-145	268.5	2.5					
S3	12.22	145	270.5	5.8					
S4	10.21	145-146	275.1	5.9					
S5	10.82	146-150	278.6	8.7					
S6	8.66	150-157	295.0	13.8					
S7	9.29	157-161	295.4	17.6					
S8	4.10	Residue	308.0	19.2					
	96.71								

## METHYL ESTERS OF "LIQUID" ACIDS L.

Primary fractionation.					Refractionation.				
No.	Grms.	B.pt./ 1 mm. °C.	Saponi- fication equiva- lent.	Iodine value.	No.	Grms.	B.pt./ 1 mm. °C.	Saponi- fication equiva- lent.	Iodine value.
L1	20.56	40-136		36.1	L11	1.10	70	174.9	3.9
					L12	1.34	70-75	188.1	7.6
					L13	1.40	75-95	196.6	8.5
					L14	1.78	95-105	231.1	13.0
					L15	3.29	105-120	237.2	23.8
					L16	3.23	120-122	251.2	32.9
					L17	1.74	122-133	268.3	35.0
					L18	4.11	Residue	296.2	74.4
						17.99			
L2	7.39	136	277.6	71.1					
L3	12.06	136-144	287.5	80.6					
L4	9.94	144-146	288.4	88.3					
L5	9.17	146-150	292.1	88.7					
L6	8.18	150	295.4	93.2					
L7	9.32	Residue	311.7	95.6					
	76.62								

TABLE II.

*Summarised Data for the Fatty Acids of Buffalo and Cow Ghee.*

Acid.	Acids non-volatile in steam.			Total. Grms.	Per cent. (excluding unsaponi- fiable matter).
	Volatile acids. Grms.	"Solid" acids. Grms.	"Liquid" acids. Grms.		
<i>Buffalo Ghee No. 1.</i>					
	29.8	263.0	178.7	471.5	
Butyric .. .. .	18.6	—	—	18.6	3.9
Caproic .. .. .	8.1	—	—	8.1	1.7
Caprylic .. .. .	1.0	—	0.6	1.6	0.3
Capric .. .. .	—	—	4.1	4.1	0.9
Lauric .. .. .	1.8	0.4	7.3	9.5	2.0
Myristic .. .. .	0.3	14.0	19.7	34.0	7.2
Palmitic .. .. .	—	93.2	0.8	94.0	20.0
Stearic .. .. .	—	114.9	—	114.9	24.4
Arachidic .. .. .	—	12.6	—	12.6	2.7
Oleic .. .. .	—	27.2	133.5	160.7	34.3
Linoleic .. .. .	—	—	12.4	12.4	2.6
Unsaponifiable matter ..	—	0.7	0.3	1.0	—
<i>Buffalo Ghee No. 2.</i>					
	14.1	122.6	93.9	230.6	
Butyric .. .. .	9.4	—	—	9.4	4.1
Caproic .. .. .	3.1	—	—	3.1	1.4
Caprylic .. .. .	1.2	—	0.9	2.1	0.9
Capric .. .. .	0.1	—	3.9	4.0	1.7
Lauric .. .. .	—	0.8	5.8	6.6	2.8
Myristic .. .. .	—	14.4	8.9	23.3	10.1
Palmitic .. .. .	—	70.8	0.8	71.6	31.1
Stearic .. .. .	—	24.5	1.1	25.6	11.2
Arachidic .. .. .	—	2.0	—	2.0	0.9
Oleic .. .. .	0.3	10.1	65.9	76.3	33.2
Linoleic .. .. .	—	—	6.0	6.0	2.6
Unsaponifiable matter ..	—	—	0.6	0.6	—
<i>Cow Ghee No. 1.</i>					
	28.4	244.9	206.1	479.4	
Butyric .. .. .	15.6	—	—	15.6	3.3
Caproic .. .. .	9.9	—	—	9.9	2.1
Caprylic .. .. .	1.4	—	3.2	4.6	1.0
Capric .. .. .	1.2	—	10.1	11.3	2.3
Lauric .. .. .	—	1.0	16.9	17.9	3.7
Myristic .. .. .	—	22.8	5.2	28.0	5.8
Palmitic .. .. .	—	143.5	—	143.5	30.0
Stearic .. .. .	—	53.6	—	53.6	11.2
Oleic .. .. .	0.3	24.0	145.6	169.9	35.5
Linoleic .. .. .	—	—	24.6	24.6	5.1
Unsaponifiable matter ..	—	—	0.5	0.5	—
<i>Cow Ghee No. 2.</i>					
	8.3	72.3	63.9	144.5	
Butyric .. .. .	3.7	—	—	3.7	2.6
Caproic .. .. .	2.8	—	—	2.8	1.9
Caprylic .. .. .	0.8	—	1.2	2.0	1.4
Capric .. .. .	0.8	—	4.3	5.1	3.6
Lauric .. .. .	—	1.7	6.6	8.3	5.7
Myristic .. .. .	—	13.2	2.1	15.3	10.6
Palmitic .. .. .	—	41.8	0.2	42.0	29.1
Stearic .. .. .	—	9.6	—	9.6	6.7
Oleic .. .. .	0.2	6.0	42.9	49.1	34.0
Linoleic .. .. .	—	—	6.4	6.4	4.4
Unsaponifiable matter ..	—	—	0.2	0.2	—



The figures for the unsaturated acids in cow ghee No. 1 are somewhat higher than are demanded by the iodine value of the original ghee, whilst the palmitic and stearic acid contents given for buffalo ghee No. 1 are markedly different from those of the three other samples; unfortunately, lack of material precluded us from repeating the analyses, as we should have preferred to do. In view of the definitely proven presence of arachidic acid in buffalo ghee No. 1, it may well be that the palmitic and stearic acid values given are not far removed from the truth; but there is almost certainly a small experimental error in the figures given for cow ghee No. 1 oleic and linoleic acids. Since, however, the rest of these respective analyses is, to the best of our knowledge, of the ordinary order of accuracy for this type of determination, and since detailed analyses of ghee fats are not numerous, we feel that, with these reservations, it is well to publish all four sets of results in order to give a general picture of the fatty acid components present in representative Indian ghees.

THE COMPONENT GLYCERIDES OF BUFFALO AND COW GHEE.—The same method was followed as in the former paper, namely, oxidation of the ghee (1 part) dissolved in acetone (10 parts) with finely-powdered potassium permanganate (4 parts), followed by isolation and purification of the fully-saturated glycerides, which alone are left unattacked. The composition of the fatty acids present in the fully-saturated glycerides was determined by the same means as were employed in the case of the original ghees, and the resulting data permit conclusions to be drawn as to the distribution of the saturated fatty acids in the fully-saturated and mixed saturated-unsaturated glycerides of the fats and as to the general composition of the mixed saturated-unsaturated glycerides.

For this part of the investigation the pasture-fed buffalo and cow ghee only were utilised, owing to shortage of material in the case of the other samples.

*Buffalo Ghee No. 2.*—Six hundred grms. of the buffalo ghee gave 213.2 grms. of crude fully-saturated glycerides (iodine value nil) which was still somewhat contaminated with acidic glyceride products of oxidation; the crude product (212.0 grms.) was further purified by boiling with dilute potassium carbonate solution, and then repeatedly with water (the aqueous and alkaline washings being extracted with ether), and yielded:

- (a) 151.5 grms. completely neutral fat, sap. equiv. 233.5 (acid value nil);
- (b) 36.7 grms. fat extracted by ether, sap. equiv. 225.2 (acid value 3.5);
- (c) 17.5 grms. acidic material, sap. equiv. 199.0 (acid value 108.7).

Assuming that the acidic matter present in (b) has the same acid value as (c), the proportion of fully-saturated glycerides in the original fat is 32.3 per cent. Analysis of the acids present in the completely neutral product (a) gave results which are summed up in Table III.

TABLE III.

Acid.	Acids.			Per cent. (excluding unsaponi- fiable matter).
	Volatile	Non-volatile	Total.	
	acids. Grms.	in steam. Grms.	Grms.	
Butyric .. ..	12.6	125.8	138.4	
Caproic .. ..	7.7	—	7.7	5.6
Caprylic .. ..	3.6	—	3.6	2.6
Capric .. ..	0.6	—	0.6	0.4
Lauric .. ..	0.7	1.0	1.7	1.2
Myristic .. ..	—	5.5	5.5	4.0
Palmitic .. ..	—	13.0	13.0	9.4
Stearic .. ..	—	74.7	74.7	54.0
Arachidic .. ..	—	29.5	29.5	21.3
		2.1	2.1	1.5

Table IV shows the general composition of 100 parts of the glycerides of buffalo ghee No. 2, as indicated by the foregoing analyses.

TABLE IV.

Glyceryl residue.	Original ghee. 100	Fully- saturated glycerides. 32.3	Mixed saturated- unsaturated glycerides (by difference). 67.7	
Glyceryl residue ..	5.0	1.7	3.3	
Butyric acid ..	3.9	1.7	2.2	(Molecular ratios)
Caproic .. ..	1.3	0.8	0.5	25
Caprylic .. ..	0.9	0.1	0.8	4
Capric .. ..	1.6	0.4	1.2	5
Lauric .. ..	2.7	1.2	1.5	7
Myristic .. ..	9.6	2.9	6.7	8
Palmitic .. ..	29.6	16.5	13.1	30
Stearic .. ..	10.6	6.5	4.1	51
Arachidic .. ..	0.8	0.5	0.3	14
Oleic .. ..	31.5	—	31.5	1
Linoleic .. ..	2.5	—	2.5	112
				9

From these figures, and also from the saponification equivalent and proportion of the fully-saturated glycerides, together with the equivalent of the original ghee and the known proportion of unsaturated acids therein present, it follows that approximately 1.2 mols. of saturated acids are linked with 1 mol. of unsaturated acids in the form of saturated-unsaturated (+ completely unsaturated) glycerides. Consequently, of 100 mols. of buffalo ghee No. 2, about 34 consist of fully-saturated glycerides, and about 42–54 of mono-unsaturated-disaturated glycerides, according as the amount of di-unsaturated-monosaturated glycerides lies between 24 and nil, and that of tri-unsaturated glycerides between nil and 12. The general structure of the buffalo ghee, allowing for its particular content of oleic and linoleic acids, is closely comparable with that of the cow butter fats recorded in the former paper; as in the latter, all the saturated acids are distributed more or less evenly throughout both the fully-saturated and the mixed saturated-unsaturated parts of the fat,

with perhaps a slight tendency for the lower fatty acids to be associated more with oleic than with palmitic or stearic acid.

*Cow Ghee No. 2.*—Six hundred grms. of the cow ghee gave 208.7 grms. of crude fully-saturated glycerides (iodine value nil), which, on further purification as previously described (p. 166), yielded:

- (a) 166.7 grms. neutral fat, sap. equiv. 245.1 (acid value 1.7);
- (b) 28.0 grms. fat extracted by ether, sap. equiv. 236.9 (acid value 8.9);
- (c) 14.0 grms. acidic material, sap. equiv. 172.2 (acid value 121.5).

Assuming that the acidic matter still present in (b) has the same acid value as (c), the proportion of fully-saturated glycerides in the original fat is 31.7 per cent.

Analysis of the acids present in the neutral product (a) gave the results summarised in Table V.

TABLE V.

Acid.	Volatiles acids. Grms.	Acids non-volatile in steam. Grms.	Total. Grms.	Per Cent. (excluding unsaponifiable matter).
	8.9	96.5	105.4	
Butyric .. ..	4.7	—	4.7	4.4
Caproic .. ..	2.8	—	2.8	2.7
Caprylic .. ..	0.4	—	0.4	0.3
Capric .. ..	1.0	2.6	3.6	3.4
Lauric .. ..	—	5.8	5.8	5.5
Myristic .. ..	—	16.8	16.8	16.0
Palmitic .. ..	—	52.2	52.2	49.6
Stearic .. ..	—	19.1	19.1	18.1

Table VI shows the general composition of 100 parts of the glycerides of cow ghee No. 2, as indicated by the foregoing analyses.

TABLE VI.

	Original ghee. 100	Fully- saturated glycerides. 31.7	Mixed saturated- unsaturated glycerides (by difference). 68.3	(Molecular ratios)
Glyceryl residue	5.1	1.6	3.4	
Butyric acid ..	2.5	1.3	1.2	13
Caproic .. ..	1.8	0.8	1.0	9
Caprylic .. ..	1.3	0.1	1.2	8
Capric .. ..	3.4	1.0	2.4	14
Lauric .. ..	5.4	1.7	3.7	19
Myristic .. ..	10.1	4.8	5.3	23
Palmitic .. ..	27.6	14.9	12.7	50
Stearic .. ..	6.3	5.5	0.8	3
Oleic .. ..	32.3	—	32.3	114
Linoleic .. ..	4.2	—	4.2	15

From these figures, or from the saponification equivalent and proportion of fully-saturated glycerides, together with the equivalent of the original ghee and the known proportion of unsaturated acids therein present, it follows that approximately 1.1 mols. of saturated acids are linked with 1 mol. of unsaturated acids in the form of saturated-unsaturated (+completely unsaturated) glycerides. So that, of 100 mols. of cow ghee No. 2, about 33 consist of fully-saturated glycerides, and about 38-53 of mono-unsaturated-disaturated glycerides, according as the amount of di-unsaturated-mono-saturated glycerides lies between 29 and nil, and that of tri-unsaturated glycerides between nil and 14. The general structure of the cow ghee is again similar in type to that of the New Zealand butter-fats dealt with in the previous paper, the saturated acids being for the most part more or less evenly distributed in both the fully-saturated and the mixed saturated-unsaturated glycerides of the fat.

#### SUMMARY AND DISCUSSION OF RESULTS.

(i) GENERAL ANALYTICAL CHARACTERISTICS AND DETAILED FATTY ACID COMPOSITION.—The two specimens of cow ghee, respectively from stall-fed and pasture-fed animals, have given figures which in each case lie within the limits of the eight New Zealand and English cow butter-fats which have been given by Hilditch and Jones (*loc. cit.*), and by Hilditch and Sleightholme (*Biochem. J.*, 1930, 24, 1098). The Reichert-Meissl values and the observed percentages of butyric acid tend perhaps towards the lowest values obtained in the New Zealand and English series, whilst the oleic acid contents lie about half-way between the extreme values recorded for the latter series. Beyond this, the milk-fat of the Indian cow, judging from these two observations, would appear very closely to resemble that of English or New Zealand cattle. The amount of stearic acid (always a somewhat variable component) is higher in the fat from the stall-fed than in that from the pasture-fed sample, whilst the palmitic acid figure (29-30 per cent.) is of the order which seems to be specific both for cow butter-fats and beef tallows.

The corresponding specimens of buffalo ghee have given figures not greatly differing from those of cow butters, although certain features seem to be distinctive. There is definitely more butyric acid present than in the average cow butter-fat from any locality, and this is reflected in somewhat higher Reichert-Meissl and Kirschner values; the proportion of linoleic to oleic acid is lower than in cow milk-fats, and the mean unsaturation (iodine value) is also lower than for the average cow butter-fats. The most apparent difference in the milk-fats of the two types of animal seems, however, to lie in the increased amount of stearic acid, and the presence of small but definite amounts of arachidic acid, in buffalo as compared with cow butters; it is also probable that the proportion of palmitic acid is more variable in buffalo than in cow butters. It is, of course, not safe to generalise too far from the examination of only two specimens of buffalo ghee, but, on the other hand, it may be remarked that our experience of cow milk fats (ten specimens of which have now been submitted to detailed study by the present

methods) leads us to believe that, broadly speaking, the main characteristics of fatty acid composition are fairly well defined for a given species of animal, subject to minor variations caused by differences in climatic or feeding conditions.

(ii) GLYCERIDE STRUCTURE.—The present results merely reinforce our previous conclusion, that the glyceride structure of milk-fats, like that of tallow and other animal fats, is of the mixed "heterogeneous" type, and is determined by factors which are independent of the particular fatty acids present. We have recently shown (*Proc. Roy. Soc.*, 1930, **129**, *A*, 468) that the relationship between the fully-saturated glycerides present in such fats and the ratio of saturated to unsaturated acids in the mixed fatty acids thereof is closely similar to that in triglycerides prepared synthetically from glycerol by heating with excess of a mixture of saturated and unsaturated fatty acids.

The pasture-fed buffalo and cow ghee, which were investigated from this standpoint, conform with this generalisation, the proportions of fully-saturated glycerides present (respectively 32.3 per cent. and 31.7 per cent.) being those which would be expected from the mean unsaturation of each fat. There has been no evidence of segregation of any fatty acid into a simple triglyceride, and all the saturated fatty acids are found, in more or less the same distribution, in each part of the fat—fully-saturated and mixed saturated-unsaturated triglycerides. It is doubtful whether triolein is present in appreciable proportions, but tripalmitin or tristearin is only present, if at all, in very small amount, and there is no reason to suppose that the quantity of triolein will be much greater.

It may be repeated, finally, that the four fats studied in this work represent first quality Indian ghees equivalent in flavour and odour to the best European or Australasian butter-fats.

We wish to acknowledge the grant to one of us (R. B.) of a Research Scholarship by the Government of the Central Provinces of India during the course of this work, and to express our appreciation of the provision of the samples of ghee by the Director of Industries, Central Provinces, Nagpur.

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## A New Method of Reduction of Tin and Antimony Prior to Titration.\*

By B. S. EVANS, M.C., Ph.D., F.I.C.

UP to the present the agents in use for the reduction of tin to the stannous condition appear to be exclusively metals, no soluble reducing agent apparently being known, or, at any rate, used. A large number of metallic reducing agents have been tried with varying success, of which, perhaps, the best is lead (A. R. Powell, *J. Soc. Chem. Ind.*, 1918, 37, 287T.). They, one and all, however, suffer from certain drawbacks which are inherent in their metallic condition, and of which the principal one is due to the fact that any metal which will reduce tin will also reduce certain other metals, notably antimony, to the metallic condition; this reduced antimony, if present in any amount, will not only completely obscure the end-point, but will also withdraw a certain proportion, too large to be neglected, of tin from solution (K. Järvinen, *Z. anal. Chem.*, 1923, 62, 184; S. G. Clarke, *ANALYST*, 1931, 82). Some metals in use, *e.g.* zinc, reduce the tin itself to the metallic condition, necessitating re-solution, and the tin deposited on the reducing metal may hinder further reduction (Oesterheld and Honegger, *Helv. Chim. Acta*, 1919, 2, 398; *ANALYST*, 1919, 44, 359). Iron, which is very commonly used, has certain disadvantages, pointed out by K. Sandved (*ANALYST*, 1927, 52, 2), and is, in addition, very liable to contain disturbing impurities; nickel has been stated to give very incomplete reduction (A. Jilek, *Chem. Listy*, 1923, 17, 223, 268, 295).

**HYPOPHOSPHOROUS ACID AS REDUCING AGENT.**—In the course of an investigation into the reduction of antimony by hypophosphorous acid it was noted that whereas, alone, antimony gave correct figures on titration, in the presence of tin, the results were very much too high, pointing obviously to the partial reduction of the tin. More careful titrations, carried out in an atmosphere of carbon dioxide, however, failed to give the full figure for the tin, 0.05 gm. of tin giving a titration of 5.75 c.c. of *N*/10 iodine, instead of the theoretical 8.42 c.c. Various catalysts were tried, and successful reduction was accomplished by the addition of a trace of mercury to the reducing solution. The peculiar reducing properties of hypophosphorous acid make it unique as an analytical reducing agent; whereas in strongly acid (say, 1:1 conc. HCl) boiling solution it is capable, as has been seen, of reducing tin to the stannous condition, yet, in cold solution of one-fifth of this acid strength, its reducing power is so low that an iodine titration of, say, reduced tin can be carried out in it without any interference from the hypophosphorous acid, beyond a tendency towards slow fading

\* Communication from the Research Department, Woolwich.

of the end-point. This being the case, a method was worked out on the following broad lines:

- (a) The tin was reduced by boiling in a carbon dioxide atmosphere with a solution of sodium hypophosphite in hydrochloric acid (1:1) and a little mercuric chloride.
- (b) The solution, after cooling, was diluted with  $2\frac{1}{2}$  times its volume of cooled boiled-out water containing citric acid, starch and potassium iodide.
- (c) The resulting mixture was titrated with standard iodine solutions.

The tin solution from start to finish was kept in an atmosphere of carbon dioxide.

APPARATUS.—It should not be necessary at this time to insist on the desirability of conducting titrations of tin entirely in an inert atmosphere; this point has been stressed many times (*e.g.* by A. Boller, Diss. Zurich, 1915; K. Sandved, *loc. cit.*; S. G. Clarke, *loc. cit.*); and if, as is the case, the operation can be carried out as quickly and with very little more trouble than the direct titration in air, it seems obviously preferable to use a method which can be performed at one's leisure, which involves no "personal factor," and which employs the theoretical factor for calculation, rather than one which relies on standardisation against metallic tin as a method of correction for a host of errors, which are necessarily always varying with the conditions. The apparatus used in this work was that which I described in a former paper (ANALYST, 1927, 52, 570), and, for convenience of reference, the description and figure are repeated here. The apparatus consists of a 750 c.c. Erlenmeyer flask, the mouth of which is closed by a three-holed rubber stopper carrying the following attachments:

- (a) A leading tube, connected by about 2 feet of rubber tubing with a Kipp's apparatus delivering carbon dioxide, and passing down to within about  $\frac{1}{2}$  inch above the liquid in the flask.
- (b) A small tapped funnel with its stem bent twice, so that the bulb of the funnel clears the burette, which is subsequently inserted in the third hole.
- (c) A removable glass plug.

The reduction is carried out in the flask, the air being displaced by a stream of carbon dioxide, which passes in through the leading tube and out through the funnel, the tap of which is left open; the glass plug is left in position. When reduction is complete, the tap of the funnel is closed, that of the Kipp's apparatus fully opened, and the flask is cooled under pressure of the carbon dioxide in the Kipp's apparatus. When cool, the glass plug is removed, the tap of the Kipp's apparatus being simultaneously regulated to deliver a steady stream, and any desired reagent admitted through the tapped funnel, care being taken not to admit any air. Finally, a burette with a jet sufficiently long to pass completely through the stopper is inserted in the hole which carried the glass plug, and the titration is carried out. In the process described below the time of reduction ( $\frac{1}{4}$  hour)

is so short that it is very improbable that the air would be adequately removed from the apparatus; consequently, the following procedure was adopted:

The stopper is removed from the flask, which is then completely filled with water; the glass plug is removed from the stopper, which is allowed to fall by its own weight into the neck of the flask, and when the water overflows through the empty hole the glass plug is re-inserted. On then pressing the stopper home the water is forced up the leading tube, which it should completely fill; if it does not it is made to do so by gently blowing into the funnel, the tap being open; the tap of the Kipp's apparatus is partly opened to allow a rapid stream of carbon dioxide to pass through the rubber connecting tube, which is then attached to the water-filled leading tube, so that air is completely excluded from the system. The apparatus is next inverted over a sink, the tap of the funnel being left open and the water allowed to run out, its place being taken by carbon dioxide. When all the water has been expelled the tap of the funnel is turned off, and the apparatus is ready for use.

DESCRIPTION OF PROCESS.—It must be emphasised that the acid strength of the solution during the reduction must not be much below 1:1 hydrochloric acid. The solution of the tin in about 30 c.c. of dilute (1:1) hydrochloric acid is run into the funnel of the apparatus, 1 c.c. of saturated mercuric chloride solution is added, and the liquid run into the flask by withdrawing the glass plug and opening the tap, care being taken not to let the surface of the liquid fall below the tap. About 5 grms. of sodium hypophosphite are placed in the beaker which contained the tin solution and rinsed into the apparatus with successive quantities of hydrochloric acid (1:1) until the total volume in the flask is about 100 c.c., the last addition being allowed to run through the tap and to within about an inch from the end of the funnel stem; the plug is now re-inserted and the tap opened, when the remaining few drops of acid are blown up into the funnel and a stream of carbon dioxide passes through the apparatus. The flask is next placed on the hot plate and the liquid allowed to boil for 15 minutes; meanwhile, 250 c.c. of water, 20 c.c. of citric acid solution (100 grms. of citric acid dissolved in 200 c.c. of water), 10 c.c. of potassium iodide solution (4 per cent.), and a few c.c. of fresh starch solution are boiled for 10 minutes in a separate flask and then cooled. At the end of the 15 minutes' reduction the tap of the funnel of the apparatus is closed, the flask removed from the plate, and the tap of the Kipp's apparatus fully opened; the flask is now cooled, the place of the contracting vapour being taken by carbon dioxide from the Kipp's apparatus. The tap of the Kipp's apparatus having been partially closed, so that only a steady stream of bubbles will pass, the plug is withdrawn and the boiled-out water, containing citric acid, potassium iodide and starch, is run into the flask, the burette containing standard iodine is inserted into the hole formerly occupied by the plug, and the solution is titrated.

For calculating the amount of tin present the theoretical factor, 1 c.c. of  $N/10$  iodine = 0.005935 grm. of tin, is used. When  $N/100$  iodine solution is employed,



a small blank, found experimentally, and representing the amount of iodine required to colour the starch, is deducted; for the present work the blank was found to be 0.2 c.c.

**TEST RESULTS.**—The following results were obtained with known amounts of tin in the form of stannic chloride:

Tin taken. Grm.	Titration. c.c.	Tin found. Grm.
0.0500	8.46 of $N/10$	0.0502
0.0400	6.72    "	0.0399
0.0300	5.02    "	0.0298
0.0200	3.48    "	0.0207
0.0100	1.74    "	0.0103
0.0050	8.6—0.2=8.4 of $N/100$	0.0050
0.0040	6.8—0.2=6.6    "	0.0039
0.0030	5.3—0.2=5.1    "	0.0030
0.0020	3.5—0.2=3.3    "	0.0020
0.0010	1.9—0.2=1.7    "	0.0010
Blank	0.2    "	

It was found that iron and copper must be separated and that nitric acid must be absent. These separations offer no great difficulty, and it is hoped shortly to publish a new method of effecting them. With regard to antimony, it was found that by increasing the amount of potassium iodide, accurate titrations of tin could be obtained in presence of a relatively large excess of antimony. The amount of potassium iodide used in the following experiments was 20 c.c. of 4 per cent. solution; larger amounts of antimony would probably require more:

Tin taken. Grm.	Antimony taken. Grm.	Titration. c.c. of $N/100$ .	Tin found. Grm.
0.0050	0.0500	8.5—0.2=8.3	0.0049
0.0040	0.0500	7.0—0.2=6.8	0.0040
0.0030	0.0500	5.2—0.2=5.0	0.0030
0.0020	0.0500	3.6—0.2=3.4	0.0020
0.0010	0.0500	1.9—0.2=1.7	0.0010

**ANTIMONY.**—As might be expected, the reduction of antimonious salts takes place much more readily than that of stannous salts, no mercury catalyst is required, and only five minutes' boiling in an open flask are needed. The antimony, dissolved in 60 to 70 c.c. of hydrochloric acid (1:1), is boiled gently for five minutes, after the addition of 5 grms. of hypophosphite, and is then cooled, after which 20 c.c. of citric acid solution (100 grms. in 200 c.c. of water) and a fragment of litmus paper are added. It is diluted with 100 c.c. of water, made alkaline with ammonia, and then slightly acid with hydrochloric acid, and again completely cooled; sodium bicarbonate is now added in decided excess (the litmus paper should be blue, and further addition of bicarbonate should not cause liberation of any more gas), followed by starch solution, and the liquid titrated with standard iodine solution (addition of potassium iodide is undesirable),

1 c.c. of  $N/10$  iodine = 0.00609 grm. of antimony.

Experiments carried out with known amounts of antimony gave the following results:

Antimony added. Grm.	Titration. c.c.	Antimony found. Grm.
0.1000	16.50—0.05=16.45 of N/10	0.1002
0.0900	14.80—0.05=14.75 "	0.0898
0.0800	13.20—0.05=13.15 "	0.0801
0.0700	11.60—0.05=11.55 "	0.0703
0.0600	9.95—0.05= 9.90	0.0603
0.0500	8.20—0.05= 8.15	0.0496
0.0400	6.60—0.05= 6.55	0.0399
0.0300	5.00—0.05= 4.95	0.0301
0.0200	3.30—0.05= 3.25	0.0198
0.0100	1.70—0.05= 1.65	0.0100
0.0060	10.15—0.40= 9.75 of N/100	0.0059
0.0040	7.00—0.40= 6.60	0.0040
0.0020	3.60—0.40= 3.20	0.0019
0.0010	2.00—0.40= 1.60	0.0010
0.0100	17.00—0.40=16.60	0.0100
0.0080	13.60—0.40=13.20	0.0079

As in the case of tin, the blank deducted from titrations was found by experiment.

Trials were made of the titration carried out in the presence of various other metals:

**LEAD.**—The only alteration made in the process was that 10 c.c. of dilute (1:3) sulphuric acid were added before reduction.

Antimony added. Grm.	Lead added. Grm.	Titration. c.c.	Antimony found. Grm.
0.0020	0.22	3.7 — 0.4 = 3.3 of N/100	0.0020
0.0040	0.22	7.1 — 0.4 = 6.7 "	0.0041
0.0200	0.22	3.38—0.05= 3.33 of N/10	0.0203
0.0400	0.22	6.66—0.05= 6.61 "	0.0403
0.1000	0.22	16.42—0.05=16.37 "	0.0997

**TIN, CADMIUM, AND BISMUTH.**—The only alteration made was, that after dilution and addition of starch, and prior to the addition of citric acid and neutralisation, N/10 iodine was added in amount required to produce a blue colour lasting for a few seconds.\* If this blue colour had not completely faded after the lapse of 3 or 4 minutes, the liquid was gently warmed until colourless. The citric acid was then added, followed by neutralisation, and the process finished as usual.

Antimony added. Grm.	Other metal added. Grm.	Titration. c.c.	Antimony found. Grm.
0.0010	0.20 Tin	2.2 — 0.4 = 1.8 of N/100	0.0011
0.0020	0.20 "	3.7 — 0.4 = 3.3 "	0.0020
0.0040	0.20 "	7.0 — 0.4 = 6.6 "	0.0040
0.0200	0.20 "	3.33—0.05= 3.28 of N/10	0.0200
0.0400	0.20 "	6.66—0.05= 6.61 "	0.0403
0.1000	0.20 "	16.47—0.05=16.42 "	0.1000
0.0020	0.10 Cadmium	3.7 — 0.4 = 3.3 of N/100	0.0020
0.0040	0.10 "	6.9 — 0.4 = 6.5 "	0.0040
0.0200	0.10 "	3.28—0.05= 3.23 of N/10	0.0197
0.0400	0.10 "	6.56—0.05= 6.51 "	0.0396
0.1000	0.10 "	16.57—0.05=16.52 "	0.1006
0.0020	0.13 Bismuth	3.7 — 0.4 = 3.3 of N/100	0.0020
0.0040	0.13 "	6.9 — 0.4 = 6.5 "	0.0040
0.0400	0.13 "	6.66—0.05= 6.61 of N/10	0.0403

\*It is desirable always to do this when tin is present as an impurity.

**ARSENIC.**—In this case the arsenic is precipitated in the form of element by the hypophosphorous acid; the following procedure was adopted:

The reduction was carried out in 30 c.c. of dilute (1:1) hydrochloric acid; the arsenic was filtered off and washed, first with 30 c.c. of dilute (1:1) hydrochloric acid, then once or twice with 5 per cent. ammonium chloride solution. The filtrate was neutralised, after addition of citric acid, as in the usual procedure, and titrated.

Antimony added. Grm.	Arsenic added. Grm.	Titration. c.c.	Antimony found. Grm.
0.0020	0.019	3.65—0.40= 3.25 of N/100	0.0020
0.0040	0.019	6.80—0.40= 6.40	0.0040
0.0200	0.019	3.30—0.05= 3.25 of N/10	0.0198
0.0400	0.019	6.60—0.05= 6.55	0.0399
0.1000	0.019	16.40—0.05= 16.35	0.0996

If, as is usually the case, the antimony, prior to titration, has to be brought into solution from a precipitate containing sulphur (this applies to the antimony precipitated by hydrosulphite as well as to the sulphide, since the liquid used for washing the former contains hydrosulphite, B. S. EVANS, ANALYST, 1929, 54, 396), it is unsafe to effect this by treatment with bromine and hydrochloric acid, owing to the likelihood of formation of sulphur bromide, which causes bad end-points and very high results in the final titration. In such cases the precipitate can be dissolved by treatment of the filter in a beaker with 10 c.c. of 20 per cent. sodium hydroxide solution and 10 c.c. of hydrogen peroxide, warming slightly and stirring thoroughly and then making strongly acid with citric acid; in the presence of some other sulphides a little dilute sulphuric acid may also be required. When the precipitate has completely dissolved, the pulp is filtered off on a small pulp filter and washed with hot water. The filtrate is boiled down to about 30 c.c., an equal volume of hydrochloric acid added, and the solution transferred to a flask and rinsed in with sufficient dilute (1:1) hydrochloric acid to bring the total bulk up to 80 to 90 c.c. The reduction and titration are then carried out as usual, omitting the final addition of citric acid.

The following table shows results obtained from lead-antimony or lead-cadmium-antimony alloys, both by the Györy titration and by the present method, following precipitation of the antimony by hydrosulphite (B. S. EVANS, *loc. cit.*):

Bromate* titration. (Antimony.) Per Cent.	Hypophosphite reduction followed by iodine titration. (Antimony.) Per Cent.
2.06 }	2.09
2.08 }	
0.54 }	0.53
0.20 }	0.20
3.06 }	3.07
3.08 }	
6.55 }	6.57
0.514 }	0.516
7.10 }	7.12
7.04 }	6.90

\* S. G. Clarke's modification, to be published shortly.

The advantages of the method here described, as compared with the bromate titration, are three in number:

- (a) Since  $N/100$  iodine can be employed, considerably smaller amounts of antimony can be titrated.
- (b) Since the reducing agent need not be removed, the boiling necessary to expel sulphur dioxide can be eliminated.
- (c) Arsenic is automatically precipitated and can be simply filtered off.

With regard to (c), work to be published shortly by S. G. Clarke shows that it is practically impossible to eliminate arsenic by boiling down the hydrochloric acid solution, as directed in Rowell's modification of the Györy process, without at the same time losing an appreciable amount of antimony; if the drop adhering to the cover glass during solution of the metal in bromine hydrochloric acid is tested, antimony is always to be found in it. In this respect, therefore, the present method seems to be an advance.

## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### THE EXTRACTIVES OF WHISKEY.

THE amount of extractives in whiskey is usually a relatively minor matter, though it has acquired official recognition in the *United States Pharmacopoeia*, X, where *Spiritus Frumenti* is required to conform to the following test: that the weight of the residue from 20 c.c. should not exceed 0.10 gm., *i.e.* whiskey should not contain more than 0.50 per cent. (w/v) of total solids.

The matter would not be worth mention here were it not for a curious statement that has become dispersed in well-known literature without receiving contradiction. In *Aids to the Analysis of Food and Drugs*, 4th edition, 1918, it is stated that "the total solids rarely exceed 0.15 per cent." The same statement occurred in the second and third editions of the book. Squire's *Companion to the British Pharmacopoeia*, 19th edition, 1916, says "the amount of extractive matter rarely amounts to more than 100 grains per gallon, equivalent to about 0.15 per cent." It appears possible that both statements were originally derived from a passage that occurred on page 106 of the first edition of Allen's *Commercial Organic Analysis*, 1879, which lays it down that "the residue left on evaporating whiskey to dryness on the water-bath should not exceed 100 grains per gallon, and is usually much less."

Allen's limit for extractives in whiskey cannot be taken as representing results to be obtained to-day. I tabulate below the weight/volume figures for total solids obtained with forty-four samples of whiskey examined since 1926, and it will be seen that in 29, or roughly two-thirds, they exceed 0.15 per cent.

Range per cent. (Grm. per 100 c.c.)	Number of samples.
0.10 to 0.15	15
0.16 to 0.20	13
0.21 to 0.30	9
0.31 to 0.40	3
0.41 to 0.53	4
Total	44

The highest figure, namely 0.53 per cent., was yielded by an adulterated sample containing 60.02 per cent. of proof spirit. It may not be out of place to suggest that on some occasions, perhaps on this, whiskey is adulterated with sherry. As the duty per degree of alcohol in wine averages 2.2d., whilst whiskey pays 8.7d., a safe and profitable adulterant would appear to be to hand in sherry.

High figures for extractives in whiskey are no novelty. A. H. Church, in his book on *Food* in 1882, refers to a case: "A sample of so-called Scotch whiskey . . . was found to be rather impure, so far as fixed matter is concerned. The total residue from one pint amounted to 50 gr., 42 of which were sugar." Fifty grains per pint amounts to 0.57 per cent. w/v.

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## THE ADDITION OF AMMONIUM SALTS TO VINEGAR.

SOME years ago certain foreign dealers were selling spurious vinegars, to which ammonium salts had been added in the correct proportion to give the analyst approximately the amount of nitrogen he would expect to find in a vinegar made from malt or from malted and unmalted grain. One of these preparations contained 60 per cent. of acetic acid, and, when diluted with eleven times its volume of water, it yielded a product containing 0.06 per cent. of nitrogen.

There has recently been a revival of this practice, and my attention has been drawn to a so-called malt vinegar which contained 0.05 per cent. of nitrogen, almost entirely derived from ammonium sulphate, presumably added in the expectation that an analyst would be unlikely to trouble about the nature of nitrogen in vinegar.

The following results, which I have obtained at various times, show that the ammoniacal nitrogen in a malt wort is usually less than a tenth of the total nitrogen, and it does not increase during acetification. It is usually rather less than the nitrogen derived from compounds "salted out" by zinc sulphate or

precipitated by bromine, and much less than the nitrogen in the compounds precipitable by phosphotungstic acid:

	Total nitrogen. Per Cent.	Precipitated by zinc sulphate. Per Cent.	Ammoniacal nitrogen. Per Cent.
Malt extract for brewing (sp. gr. 1.330)	0.42	0.098	0.021
Malt extract for brewing (sp.gr. 1.310)	0.79	—	0.032
Wort from barley malt (sp.gr. 1.077) ..	0.14	—	<sup>1</sup> 0.002
Vinegar from wort of sp.gr. 1.052 ..	0.12	0.015	<sup>2</sup> 0.015 } <sup>1</sup> 0.009 }
Medicinal malt extract .. ..	0.77	0.19	—
Malt extract for brewing (sp.gr. 1.333)	1.52	0.17	—
Commercial "malt" vinegar ..	0.089	0.008	0.007

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<sup>1</sup> Distilled with barium carbonate, after neutralisation with sodium hydroxide.

<sup>2</sup> Distilled with sodium hydroxide.

#### A SIMPLE CALCULATION OF THE LIMIT OF VALUE OF THE MICROSCOPICAL EXAMINATION OF MILK FOR TUBERCLE BACILLI.

LET us suppose that 100 c.c. of milk are centrifuged, and that the volume (V) of deposit is roughly measured; that films of the deposit are uniformly spread on cover-glass circles, (D) mm. in diameter, and that, after staining, the number (n) of tubercle bacilli per 100 fields is determined. The number (N) of tubercle bacilli per 100 c.c. of milk will be given by the equation

$$N = \frac{V}{v} \times \frac{D^2}{d^2} \times \frac{n}{100}$$

where v = the volume of deposit spread over the cover-glass circle, and d the diameter of the microscope field.

Of course, it is quite immaterial whether films are made on slides or cover-glass circles, the essential point being the volume of deposit per unit area. I find that v lies between 0.0025 and 0.005 c.c., say, 0.005 c.c., per cover-glass circle 19.05 mm. in diameter, and that with the microscope in use d=0.15 mm. Hence

$$\begin{aligned} N &= \frac{V}{0.005} \times \left( \frac{19.05}{0.15} \right)^2 \times \frac{n}{100} \\ &= V \times 32,000 \times n. \end{aligned}$$

V may vary from 0.2 c.c. to 30 c.c.

It is very obvious that unless N is very large, n will be very small, and it is only in cases of very advanced tubercular mastitis that n becomes a whole number. The reciprocal of n is of chief interest; this represents the number of times separate areas, each of 100 fields, must be examined in order to find 1 tubercle bacillus for any given values of N and V. It has been shown that a single tubercle bacillus can be detected biologically in 40 per cent. of trials, and two tubercle bacilli in 67 per cent. of trials.\* One may reasonably assume, therefore, that 5 tubercle bacilli will be detected by the biological test.

\* *The Prevention of Human Tuberculosis of Bovine Origin.* W. G. Savage, p. 131.

Taking an extreme example, and supposing that only 5 tubercle bacilli are present in 100 c.c. of milk, and taking a very moderate value for  $V = 0.5$  c.c.

$$5 = 0.5 \times 32,000 \times n = 16,000 \times n, \text{ or } \frac{1}{n} = 3,200.$$

The average time taken by three individual workers in this laboratory to view 100 fields is  $2\frac{1}{4}$  minutes; on the average, therefore, one would have to spend  $2\frac{1}{4} \times 3,200$  minutes, or 120 hours, in finding one tubercle bacillus, and an equal length of time in confirming the result; or, taking a less extreme case, and supposing 50 tubercle bacilli to be present in 100 c.c., one would have to search (on the average) for 12 hours. If we set a limit to our time of search and make this limit 36 minutes, all that we can claim, if we fail to find tubercle bacilli, is that (probably) less than 1000 are present per 100 c.c. of milk, provided  $V=0.5$  c.c.; but if the milk has an abnormal cellular content, it will give a larger deposit, and the test will be proportionately less delicate. In an extreme case of milk, ropy with pus and giving a deposit of 25 c.c., one would only be able to say that less than 50,000 tubercle bacilli were present per 100 c.c. or 500 per c.c.

Without concentration, therefore (and none of the concentration methods hitherto proposed are really successful) the microscopical examination of milk for tubercle bacilli is limited to the detection of from 10 to 500 bacilli per c.c., or, excluding those cases with bulky deposits which are not characteristic of tubercular mastitis but of tubercular mastitis complicated by streptococcal mastitis, and confining our consideration to milk giving a deposit of not more than 2.5 c.c. per 100 c.c., the examination is limited to the detection of not less than 10 to 50 bacilli per c.c., and is about 500 times less sensitive than the biological method.

SOMERSET COUNTY LABORATORY.

D. R. WOOD.

### THE FREEZING-POINT METHOD FOR THE EXAMINATION OF COCOA BUTTER.

OWING to a misunderstanding, I omitted to mention in my paper on the Classification of Chocolate Fats (ANALYST, 1930, 55, 477), that the standard solidification test there described was devised by Mr. H. R. Jensen, and that part of the experimental work cited in the paper was done while I was working in his laboratory.

A. G. AVENT.

## Official Appointments.

THE Minister of Health has confirmed the following appointments:

ERIC VOELCKER, A.R.C.S., F.I.C., as Public Analyst for the County of Northampton (January 28, 1931).

J. A. VOELCKER, C.I.E., Ph.D., F.I.C., as Additional Public Analyst for the County of Northampton (January 28, 1931).

The Minister of Agriculture and Fisheries has confirmed the following appointments:

F. E. BULLOCK, F.I.C., as Agricultural Analyst for the County Borough of Leicester (February 6, 1931).

J. A. VOELCKER, C.I.E., Ph.D., F.I.C., as Official Agricultural Analyst for the County of Northampton (February 4, 1931).

ERIC VOELCKER, A.R.C.S., F.I.C., as Deputy Agricultural Analyst for the County of Northampton (February 4, 1931); and as Deputy Agricultural Analyst for the County of Northumberland (February 18, 1931).

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### REFINED BORAX.

ON January 10th, a co-operative society was summoned at Leicester for selling borax not of the nature, substance and quality demanded.

On analysis the sample was found to contain 60 parts of arsenic per million, whereas purified borax, according to the British Pharmacopoeia, should not contain more than 5 parts per million.

For the defence it was urged that purified borax is a medicinal preparation, but that refined borax is a commercial article used for domestic purposes. On the side of the packet were the words: "Finest Refined Borax," and there was also a notice that it was not to be taken internally, but only to be used for domestic purposes.

Evidence was given that to get a poisonous dose it would be necessary to take 5 lbs. of the borax.

The Magistrates dismissed the case.

### EXCESS OF SULPHUR DIOXIDE IN CANDIED PEEL.

ON January 14, a Sheffield wholesale firm was summoned at Cardiff for selling candied peel containing sulphur dioxide to the amount of 40 parts per million in excess of that permitted by the Public Health Regulations, 1927.

The solicitor, for the defence, submitted that the summons was wrongly worded, the defendants being charged with selling candied peel, whereas only lemon peel was supplied; but the Stipendiary held that the Regulation applied to any of the three peels—lemon, orange or citron.

The General Manager of the defendant firm stated that a different process was used with orange and citron than with lemon peel. As lemon peel would not stand the same heat as the other two peels, it was necessary to add additional glucose and sugar to it, and each of these ingredients contained sulphur dioxide.

The Stipendiary imposed a fine of 40s. and costs.



## New Zealand.

### SIXTY-THIRD ANNUAL REPORT OF THE DOMINION ANALYST.

THE Dominion Analyst (Mr. W. Donovan), in his Report for 1929, states that 5462 samples were examined in the Dominion Laboratory for the different Government Departments. Of the 3788 samples examined for the Health Department, 2789 were milks. In the Christchurch Branch Laboratory, 1288 samples of milk were analysed. Of these, 1003 were for the Metropolitan area; and as there are 600 milk dealers, the sampling control is regarded as quite inadequate. The sampling at the Dunedin Branch (735 milks) was also very inadequate.

**MINERAL CONTAMINATION OF AERATED WATERS.**—Of the 153 samples of soda water examined at Wellington, lead was present in at least 51 samples, copper (traces) in 2, and iron in 9. It was noticed that soda waters from Hastings and Lower Hutt, where the waters are hard, were practically free from lead. Many of the soda waters were discoloured by material extracted from the corks.

**NEW ZEALAND WHALE OILS.**—The following results were obtained in the analysis of three samples from Whekeniu, Picton:

Acid value .. .. .	12.7	5.3	14.8
Saponification value ..	191.7	191.6	190.0
Iodine value .. .. .	134.8	139.0	140.5
Refractive index at 40° C. ..	1.4655	1.4660	1.4662
Refractive index at 60° C. ..	1.4575	1.4585	1.4588
Specific gravity at 15°/15° C.	0.923	0.925	0.923

These oils differed from Antarctic whale oils in throwing down hardly any stearine when chilled.

## Trinidad and Tobago.

### ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1929.

IN his Annual Report to the Legislative Council, the Government Analyst (Mr. H. S. Shrewsbury) states that 2760 samples were examined, including 935 milks, 862 aerated waters, 186 spirit compounds, 141 oils, and 88 butters. The percentage of adulterated food and drugs samples, which was 65.3 in 1891, and 32.5 in 1900, was 5.7, as compared with 5.8 in 1928.

**CORROSION OF WATER PIPES.**—In connection with the Island water scheme, 10 samples of earth from the route of the proposed pipe line were examined for corrosive properties, and, with the exception of those connected with the corrosion of existing pipe lines at Prince's Town, were found to be satisfactory. Samples of cast-iron pipes at Prince's Town and soil in which they were embedded, were submitted by the Public Works Department. The pipes were very badly pitted, the holes having remarkably clearly defined edges. It appeared that the piping had been corroded by contact with moist fragments of selenite or gypsum in the soil.

**TOXICOLOGICAL.**—In 52 samples (compared with 24 in 1928) of post-mortem remains and stomach washings, five poisons were discovered, namely, cresylic disinfectant, aconite, arsenic, luminal and strychnine. This was the first case of luminal poisoning in the Colony.

**POISONING OF COCONUT TREES.**—In this unusual case, samples of coconut trees which had died in suspicious circumstances, were submitted for analysis. Holes had been bored into the trunks, and evidently a solution of sodium arsenite had been poured into these, as the wood surrounding the holes contained 1 per cent. of sodium arsenite.

**LIME SKINS AS CATTLE FOOD.**—A sample of lime skins (after extraction of oil and juice) was submitted for analysis to determine their suitability as cattle food. Analysis showed this might be a suitable cattle food, but feeding experiments are advisable.

**HYDROCARPUS OIL.**—Twelve samples of hydnocarpus oil were submitted by the Surgeon-General. This oil is used in the treatment of leprosy at Chachacare. These particular samples caused intense local pain on injection. After a lengthy investigation it was found that these samples contained three impurities, any one of which might have caused the trouble.

## United States Department of Agriculture.

### REVISED AND AMENDED DEFINITIONS AND STANDARDS FOR FOOD PRODUCTS.\*

#### CORN SUGAR.

The definitions for food products in S.R.A., F.D. No. 2, and supplements thereto, were adopted prior to the departmental announcement of December 26, 1930, which reads as follows:

"Corn sugar (dextrose) when sold in packages must be labelled as such; when sold in bulk must be declared as such; but the use of pure refined corn sugar as an ingredient in the packing, preparation, or processing of any article of food in which sugar is a recognised element need not be declared upon the label of any such product.

"Nothing in this ruling shall be construed to permit the adulteration or imitation of any natural product, such as honey, by the addition of any sugar or other ingredient whatever."

The term "sugar," with or without the parenthetical expression "sucrose," as used in the definitions to designate the sweetening agent in manufactured food products, is to be interpreted, wherever necessary to effect the purpose of the foregoing decision, as including dextrose (pure, refined corn sugar).

\* Food and Drug Administration. Service and Regulatory Announcements. Food and Drug, No. 2 (First Revision). Supplement No. 3. (Dec. 31st, 1930.)

**Erratum:** *Notes on the Thiocyanate Method of Estimating Iron*, by G. W. Leeper (ANALYST, 1930, 55, 371): For "iron content," 6 lines from the end, read "phosphorus content."

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

## Food and Drugs Analysis.

**Indications of Glucose in Milk.** C. H. Whitnah. (*J. Amer. Chem. Soc.*, 1931, 53, 300-304.)—The optical rotation of milk is not, as a rule, altered by treatment with yeast, but the extra rotation imparted to milk by addition of 0.5 per cent. of glucose is completely removed by leaving 50 c.c. of the milk in contact with 3 grms. of yeast for an hour at 30° C. The yeast is prepared from bakers' yeast by washing and centrifuging five times, the final washing water being clear and free from reducing sugars. Fermentation of 275 samples of milk in this way, without added glucose, showed that the resulting decrease in rotation varied from zero in 20 cases to a value corresponding with 0.35 per cent. of glucose in the milk. No explanation is advanced for increases in rotation amounting to 0.01 to 0.07 per cent. observed with 11 other samples. The apparent presence of glucose seems to be independent of any other characteristics of the milk. T. H. P.

**Retained Milk.** O. Laxa. (*Ann. Falsificat.*, 1930, 23, 609-610.)—Twelve cows in the month of August travelled by rail for 3 days, and were not milked till they arrived at the farm, and four of these then only yielded about 100 c.c. of milk. This milk resembled colostrum, and was white with a brown tint, and had a smell of burnt milk, attributed to the decomposition of the milk sugar. The milk contained water, 78.4; fat, 10.15; casein, 2.65; albumin and globulin, 0.0; albumoses and peptones, 2.92; amino acids, 1.78; milk sugar, 1.50; ash, 1.10; and undetermined substances, 1.50 per cent. D. G. H.

**Determination of Starch in Cereal Products.** C. W. Herd and D. W. Kent-Jones. (*J. Soc. Chem. Ind.*, 1931, 50, 15-22T.)—The difficulties encountered in the application of the various hydrolytic methods proposed for the determination of starch in cereals are due mainly to the following causes: Acid acts on matter other than starch; prepared diastases vary in hydrolytic power and exert unknown action on the different components of the starch; malt diastase exhibits varying hydrolytic action on the hemicelluloses; and with barley diastase, in addition, it must be assumed that the amylose-maltose value for other starches is the same as for potato starch. A number of the best-known methods have been investigated, with results which suggest the following remarks:

*Scheele and Svensson's method* (*Tekn. Tidskr.*, see *Milling*, 1928, 518): The meaning of "diastase absolute" is not clear, but the use of 0.1 gm. of a good prepared diastase with 3 grms. of substance gave very low results, and even 0.5 gm. of the diastase gave lower results than other methods. The method is hence complicated by the necessity for determining the conditions for complete hydrolysis with each batch of enzyme. *Hartmann and Hillig's method* (ANALYST,

1927, 52, 160): A satisfactory method could probably be based on preliminary treatment for 12 hours with pepsin and subsequent hydrolysis with prepared diastase, but the necessity of modifying the procedure for different deliveries and the lack of knowledge of the effects on the "hemicelluloses" render the method unsuitable for general use, and render it difficult for analysts to check the work of others.

*Ling's Malt Extract Method* (ANALYST, 1923, 48, 29, 554).—This requires modification to facilitate the filtration and washing of the insoluble matter from the conversion liquid, and, with flours, 2 (not 5) grms. should be taken; the accuracy attained is only about  $\pm 1$  per cent., but within this limit the method works well with wheats and flours.

*Ling, Nanji and Harper's Barley Diastase Method* (J. Inst. Brew., 1924, 30, 838).—Here the accuracy is within  $\pm 0.5$  per cent., and the method seems satisfactory for wheats and flours, but takes longer; if the amylose-maltose figure for potato starch is accurate and is applicable to other starches and cereal products, the method appears sound and reliable.

The most important of the non-hydrolytic methods is that of Rask (ANALYST, 1927, 52, 290), which presents various practical difficulties. These are overcome, even with sharps and bran, by the following procedure: One grm. of the material and 1 grm. of acid-washed sand are mixed in a centrifuge tube, stirred for about one minute with washed ether, and centrifuged, the liquid being then poured off. After two repetitions of this washing, the residue is mixed with 2.5 c.c. of water and 0.25 c.c. of *N* sodium hydroxide. After about 15 minutes, 5 c.c. of pure methyl alcohol are mixed in and then 5 c.c. of dilute methyl alcohol (5 alcohol: 2.5 water). The mixture is centrifuged, the layer of alcohol removed, and the residue washed twice with 10 c.c. of the dilute methyl alcohol, and finally three times with water. The mass is next stirred to a thick paste with a few c.c. of water, all lumps being broken down. A total of 20 c.c. of water is added to effect transference to a 100 c.c. flask, 20 c.c. of concentrated hydrochloric acid being then added and the volume made up with Rask's acid (21 grms. of HCl per 100 c.c.). The latter is used to rinse the centrifuge tube. The contents of the flask are filtered with the aid of suction through a Gooch crucible, charged with asbestos and with a layer of acid-washed sand, the filtrate being collected in a small dry flask. Of the filtrate, 50 c.c. are transferred by a calibrated pipette to a 200 c.c. beaker containing 110–115 c.c. of 96 per cent. alcohol, this step being completed within 35 minutes of the initial contact of the acid with the starch, so that appreciable hydrolysis of the starch may be avoided. Immediately after the 50 c.c. pipette has drained completely, but not until then, the mixture is stirred continuously for about 1 minute, or until the precipitate has become flocculent. When the precipitate has partly settled, the whole is centrifuged for 10 minutes, the liquid being then removed and the residue washed three or four times with 70 per cent. (vol.) alcohol and twice with 96 per cent. alcohol, the starch being thoroughly mixed with the alcohol before centrifuging. The final residue is transferred to a tared Gooch crucible with 96

per cent. alcohol and washed with ether, the crucible being placed for 10–15 minutes in an oven at about 40° C., and then dried at 130° C. to constant weight. With sharps and bran, 2–4 grms. must be used, the quantities of the methyl alcohol and sodium hydroxide mixture being increased in proportion. Volume allowances may be made when using wheat, bran or sharps, or, alternatively, the crude fibre may be retained on a small pad of glass wool before making the volume up to 100 c.c.

The malt diastase, barley diastase, and modified Rask methods have been applied to: (1) *Commercial starches*: Malt diastase gives slightly higher results than barley diastase, but the Rask method gives the highest starch percentages, these, when added to those for moisture and other components, giving 98.85 and 99.60 for the two starches examined. (2) *Flours*: The malt and barley diastase results differ but little, and are appreciably higher than the Rask results; it is found that the residue of the flour not dispersed by hydrochloric acid contains substances giving reducing sugars on treatment with diastase. (3) *Wheat Offals*:—The malt and barley diastase methods give fairly concordant figures, but the Rask results are low in all cases, indicating that diastase hydrolyses substances which are not strictly starch. This is supported by the observation that fibre separated by the method recommended by the Board of Agriculture and Fisheries (Fertilisers and Feeding Stuffs Regulations, 1928), and hence free from starch, gave about 7 per cent. of reducing sugars, calculated as maltose, when treated with barley diastase. (4) *Whole wheats*:—In all cases malt diastase gives rather higher results than barley diastase, the Rask results being parallel to neither of the other two.

From these results, the authors conclude that, for cereal chemists, the modified Rask method described above is the most generally useful. T. H. P.

**Detection of Sorbitol in the Analysis of Jams.** C. F. Muttelet. (*Ann. Falsificat.*, 1930, 23, 602–605.)—Twenty grms. of fruit juice or 75 grms. of jam are dissolved in 4 to 5 parts of warm water, and a little charcoal added, and after 5 minutes' boiling the solution is filtered. The colourless filtrate is sterilised for 15 to 20 minutes at 115–120° C., and, after addition of bakers' yeast, is kept at 25–30° C. for a few days until fermentation is as complete as possible. A pinch of charcoal is added, the liquid filtered, and the filtrate concentrated *in vacuo* to a few c.c., and 1 to 2 c.c. of 50 per cent. sulphuric acid and 4 to 5 drops of freshly distilled benzaldehyde added. The corked flask is shaken for 10 to 15 minutes, and, after 12 hours' contact, a precipitate is formed corresponding to the amount of sorbitol present. To facilitate filtration, 100 c.c. of cold water are added, little by little, and after a few hours the precipitate is washed with warm water and a little alcohol, dried *in vacuo*, and weighed. The weight is about twice that of the sorbitol present. If desired, further purification may then be carried out. The use that may be made of the test may be judged from the following figures: Cherry juice contained 0.29 gm. per cent. of sorbitol, but none was present in red gooseberry or strawberry juice. The proportion found in home-made gooseberry jelly was nil, in cherry jam 0.125 per cent., and in strawberry jam, nil: but commercial

gooseberry jelly "pure fruit" contained 0.135 gm.; cherry jam "fantaisie," 0.025 gm.; and strawberry jam "pure sugar and fruit," 0.075 gm. per cent. The commercial gooseberry jelly was, in fact, gooseberry and apple (confirmed by the detection of malic acid); the cherry jam consisted of portions of cherry in sugar jellified with industrial pectin, and the strawberry jam contained strawberries in a syrup made of apple juice.

D. G. H.

**Notes on the Histology of the Almond.** V. A. Pease. (*J. Agric. Res.*, 1930, 41, 789-800.)—Four varieties of Californian almonds and one variety each from Etna, Marcona, Valencia, and Alicante were comparatively examined from a histological point of view, and the differences found indicate the possibility of developing an easily workable method for distinguishing the soft shelled Californian and hard-shelled European almonds when in the shelled condition. The cells of the outer epidermis of the testa or surface of the seed coat differ, in that those of the soft-shelled almonds are only slightly lignified compared to the others, and are to be regarded as poorly developed stone cells. Further, the stone cells of hard-shelled varieties are more elongated, the ratio of length to width being 2 to 1 against 5 to 4 in the soft-shelled almonds.

D. G. H.

**Nature of the Highly Unsaturated Fatty Acids Stored in the Lard from Pigs Fed on Menhaden Oil.** J. B. Brown. (*J. Biol. Chem.*, 1931, 90, 133-139.)—Reports by Brown and Deck (*J. Amer. Chem. Soc.*, 1930, 52, 1135) and Brown (*J. Biol. Chem.*, 1928, 80, 455) have shown that when fish oil was given to white rats, equilibrium between the diet and the deposited fat resulted in from 4 to 6 weeks, and that with a series of diets containing from 5 to 30 per cent. of menhaden oil the amount of highly unsaturated acids in the deposited fat was roughly proportional to their content in the diet. Relatively more of these acids, however, were found in the fat from animals on the diets of lower oil content. Analysis of the polybromides of the fatty acids from the body fats resulted in each case in higher bromine content than the bromides from the original fish oil acids, suggesting that the deposited acids were more unsaturated than those from the original oil. It was decided to feed a larger species of animals on a fish oil diet, with the hope of being able to isolate enough of the deposited unsaturated acids to study their properties and ascertain whether they had undergone change during assimilation. Pigs were chosen as experimental animals because they will readily eat food containing large amounts of the oil, and, further, the yield of lard is sufficient for the preparation of the highly unsaturated acids. The pigs fed on fish oil were decidedly more nervous during killing than the controls, and their carcasses were yellowish in colour. The fishy odour was scarcely noticeable in the fresh carcase, but, on cooking, it had penetrated to all parts; even the innermost parts of the tongues were highly flavoured. When the pigs were given a diet containing about 14 per cent. of menhaden oil, the lipids and fatty acids of the livers were decidedly more unsaturated than those of the controls, indicating a mobilisation of highly unsaturated acids. Under these dietary conditions there was a storage of 2.7 per cent. of highly unsaturated acids in the lard. The highly

unsaturated fatty acids deposited were of about the same molecular weight and of lower iodine value than the mixture of acids isolated in a similar manner from the original mehaden oil, and, therefore, were less unsaturated. The explanation is given that the pig burns the more highly unsaturated acids from the oil, and deposits the remainder. With rats, the deposited acids were more unsaturated than those from the oil. The author believes that the metabolisms of the pig and the rat are sufficiently different to explain the apparent contradiction of results.

P. H. P.

**Oxidation of Official Castor Oil by Potassium Permanganate. Study of Triazelain.** G. Schuster. (*J. Pharm. Chim.*, 1931, 123, 5-12.)—Castor oil, having a saponification value of 183 and an iodine value of 83, was oxidised with permanganate in acetone solution by the method of Hilditch (*J. Chem. Soc.*, 1927, 3106; *cf.* ANALYST, 1929, 54, 75), and 63.58 per cent. of crude triazelain was obtained, but neither stearic nor dihydroxystearic acids were found on saponification of this product; and when 3 per cent. of stearic acid was added to the castor oil the same amount was recovered after oxidation. It is concluded that no glycerides of saturated acids were present in the castor oil. Triazelain was synthesised by heating acid sodium azelaite and glycerin epichlorhydrin in a closed tube at 135-140° C. for 10 hours, after which the mass is taken up with hot water, the excess of epichlorhydrin removed, and the triazelain precipitated with hydrochloric acid. Triazelain is a dark yellow, oily, fluorescent liquid, insoluble in water, petroleum spirit or benzene, but very soluble in ether, strong alcohol and alkalis. It should be stored *in vacuo*. It readily saponifies, and is hydrolysed by 0.1 *N* hydrochloric acid. The sodium salt forms fine needle-shaped crystals. A colourless precipitate is formed with silver nitrate and a neutral solution of sodium triazelain, and with barium chloride, bismuth nitrate, cadmium sulphate and strontium nitrate, buff precipitates are formed. With magnesium chloride a buff precipitate of the triazelainate is formed, whilst the magnesium salt of azelaic acid under the same conditions is soluble. This allows of purification of triazelain by precipitating it as its magnesium salt.

D. G. H.

**Some Oleaginous Forest Seeds of Angola.** C. de M. Gerales, A. d'Almeida and C. Duarte. (*Bull. Mat. Grasses*, 1930, 14, 332-342.)—The seeds examined were obtained from North West Angola, and in every instance the oils were extracted with ether. *Allanblackia floribunda*.—It is considered that a variety particularly rich in oil might be developed by selection. The seeds are of a darker reddish brown than those of *Allanblackia saculeuxii*, and both trees attain a height of some 25 metres. The fat from the former species is the more important as a source of stearine. *Irvingia robur* has fruits consisting of 53.4 per cent. of epicarp and mesocarp and 46.6 per cent. of stone, and the stone is made up of 93.25 per cent. of endocarp and 6.75 per cent. of seed, which contains 7.2 per cent. of moisture, and 64.85 per cent. of fat. The seeds are not, therefore, of economical importance. *Balanites mayumbens* is a new type of *Balanites*, and has a larger proportion of seed to endocarp (48.2 to 51.8 per cent.) than other species, and its

seeds are larger—an important point, in view of the difficulty of decorticating. The oil has the same general characteristics as the oils of other species of *Balanites*. The oil of *Strimbosia Scheffleri* is of a reddish yellow brown colour and of a disagreeable taste and smell, although this may be due to the high acidity of the examples examined. The oil is consumed in small quantity by the natives, but in larger proportions it produces vomiting. The seeds of *Mimusops Ebolowensis* yield kernels with 4.75 per cent. of fat, but a very hard thick episperm is present, and the seeds are economically unimportant.

Common Name.	<i>Allanblackia</i> <i>floribunda</i> , <i>sacleuxii</i> Nua.		<i>Balanites</i> <i>mayumbens</i> .	<i>Strimbosia</i> <i>Scheffleri</i> .
	Lalanjo.	M'bunze.	Buzupundi.	M'senha.
<i>Seed.</i>				
Shell, per cent. ..	14.82	15.57	9.37	37.6
Kernel ..	85.18	84.43	90.63	62.4
<i>Kernel.</i>				
Moisture, per cent. ..	4.11	2.98	10.10	13.70
Fat, per cent. ..	68.53	72.09	39.06	15.58
<i>Fat.</i>				
M.pt., °C. ..	40.7	41.8–42.8		
Solidification point, °C. ..	39.5–40.5	41.0–42.0		
Sp.gr. at 15° C. ..	0.9187	0.9194	0.9171	0.9353
$n_D^{20}$ ..	1.4529	1.4551	1.4602	1.4716 (25°)
Saponification value ..	195.0	207.85	204.61	216.2
Iodine value ..	33.3	29.5	101.0	83.6
Acid value ..	7.5	35.3	71.12	39.1
M.pt. of fatty acids, °C. ..	—	61–63	—	—

D. G. H.

**Evaluation of Pyrethrum Flowers.** J. T. Martin and F. Tattersfield. (*J. Agric. Sci.*, 1931, 21, 115–135.)—For pyrethrum flowers rich in pyrethrins the method of determination given by Tattersfield and Hobson (*ANALYST*, 1929, 54, 351) yields low results, and is now modified in various ways. With samples containing below 0.7, 0.7–1.5, and above 1.5 per cent. of total pyrethrins, 10, 5, and 2.5 grms. are taken for analysis. These are extracted with petroleum spirit, and, after the bulk of the solvent has been evaporated in a current of carbon dioxide and the rest in a vacuum desiccator, the residue is extracted with four quantities of 2.5 c.c. each of gently warmed purified methyl alcohol, each of which is cooled and filtered through cotton wool into a 100 c.c. Kjeldahl flask. After a final washing with 2.5 c.c. of cold methyl alcohol, a few drops of methyl alcoholic phenolphthalein are added, and then, dropwise, *N* methyl alcoholic potassium hydroxide, until the liquid is just alkaline. An excess of 5 c.c. of the alkali is then added and the mixture heated in a reflux apparatus for 8 hours, the methyl alcohol being expelled in a partial vacuum at a temperature not exceeding 25° C., and the residue dissolved in water. The volatile acid is next distilled off in steam, the



volume in the distillation flask being kept below 30 c.c. Two lots of 50 c.c. are distilled off, and the acids in the first of these extracted with two 50 c.c. quantities of petroleum spirit, each extract being washed with 20 c.c. of water. The two extracts are combined, evaporated with 20 c.c. of water on a water-bath, and the residue titrated while warm with 0.02 *N* sodium hydroxide, the sides of the flask being washed down with a little neutral methyl alcohol towards the end of the titration; 1 c.c. of 0.02 *N* alkali represents 3.36 mgrms. of monocarboxylic acid or 6.6 mgrms. of pyrethrin I. The second 50 c.c. of distillate may be extracted with petroleum spirit, but should not show more than a trace of titratable acid. The hot aqueous residue in the distillation flask is treated with 0.2 gm. of calcium sulphate and filtered the next day through cotton wool, washed three or four times with water and extracted exhaustively with sodium-treated ether in the apparatus previously described (*loc. cit.*). In a rapid extractor 20 hours appears to be the minimum time necessary for complete extraction of the dicarboxylic acid with samples of high pyrethrin content. After addition of 20 c.c. of water the ether is evaporated and the aqueous layer heated to boiling, cooled and filtered through cotton wool, the filtrate being heated to boiling and titrated with 0.02 *N* sodium hydroxide; 1 c.c. of the alkali corresponds with 1.98 mgrm. of dicarboxylic acid or 3.74 mgrms. of pyrethrin II. The method requires considerable care, the determination of pyrethrin II being attended with various technical difficulties.

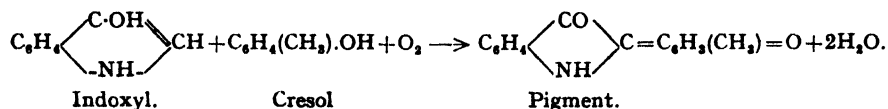
A short method for determining the total pyrethrins is given, two procedures being described, the first for use with 0.5 gm. of the powdered material, and the second with a single flower head (about 0.1 gm.). This method, which gives results in general concordance with both the acid method and the Gnadinger and Corl method (*ANALYST*, 1929, **54**, 754), depends on partial reduction of standard alkaline potassium ferricyanide solution by means of the ketone group of the pyrethrolone fraction of the pyrethrin molecule. The ferricyanide present before and after the reaction is determined by liberating its iodine equivalent and titrating with thiosulphate. Graphs are given showing the relationship between the amount of ferricyanide reduced, expressed as c.c. of 0.005 *N* thiosulphate, and the number of mgrms. of pyrethrins I and II in an aliquot portion of the final pyrethrin extract.

Good agreement is obtained between the analytical data and the results of insecticidal tests on *Aphis rumicis* T. H. P.

## Biochemical.

**Geographical Location and Iodine Content of the Thyroid Gland.** F. Fenger, R. H. Andrew and J. J. Vollersten. (*J. Amer. Chem. Soc.*, 1931, **53**, 237-239.)—The yearly average iodine content for hog glands from North Dakota (latitude 47° N., winters severe) is 0.32 per cent., whereas that for glands from Texas (33° N., winters mild) is 0.60 per cent., calculated on the desiccated fat-free basis. The seasonal variation is the more pronounced in the former case. These differences are attributed to geographical differences. T. H. P.

**The Urocarmine Reaction.** W. R. Fearon and A. C. Thompson. (*Biochem. J.*, 1930, 24, 1371-1378.)—A specimen from some miscellaneous urines was found, on treatment with concentrated hydrochloric acid and an oxidising agent, such as hydrogen peroxide or potassium chlorate, to give a carmine colour, which investigation showed arose from a condensation between indoxyl and *o*- or *m*-cresol present in the urine, in accordance with the equation:



Further investigation with various phenols of biological interest suggested that the condensation takes place between the indoxyl and the phenol in the unsubstituted *p*-position of the latter to form the leuco-base; the pigment was not obtained with any of the *p*-substituted phenol compounds examined. In order to distinguish them, therefore from other red urinary pigments, such as indirubin, urorosein and the scatole-reds, the term *urocarmine* has been applied to the class of indogenide pigments obtained from the condensation of indoxyl with various phenols unsubstituted in the *para*-position. The reaction might be of use in determining the presence or absence of *p*-substitution.

**Method of demonstration of the urocarmine test.**—This is a modification of the indigo-blue test made under conditions less favourable for the production of the blue dye; 2 c.c. of urine are treated with two drops of 0.1 per cent. potassium chlorate solution or of 3 per cent. hydrogen peroxide, and then 10 c.c. of colourless concentrated hydrochloric acid are added. The mixture is well shaken, left to stand for a few minutes, and extracted first with chloroform and then with amyl alcohol. Under these conditions most normal urines give a variable indigo-blue reaction, the pigment dissolving in the chloroform, but some specimens will be found to display a reddish colour not removed by extraction with chloroform, but very soluble in amyl alcohol. On the addition of resorcinol or *o*-cresol, followed by acidification, most, if not all, normal urines give a urocarmine reaction, and this may be made the basis of a simple colorimetric method for the determination of indoxyl. The preparation and isolation of *o*-cresol urocarmine are described. The urocarmine reaction accounts for many of the pigments obtained from urine which are insoluble in chloroform. The classification of the urocarmine reactions of urine can now be simplified.

*Classification of the red indogenide pigments.*

(A) Pigments soluble in chloroform.

*Indirubine* or indigo-red.

(B) Pigments insoluble in chloroform.

1. *Urocarmines*. Simple condensation products of indoxyl and urinary phenols unsubstituted in the *para*-position. Both chromogen and pigment are soluble in amyl alcohol. Chromogen precursors can be resolved by steam distillation into volatile and non-volatile components.

(a) *Phenol indogenide*.

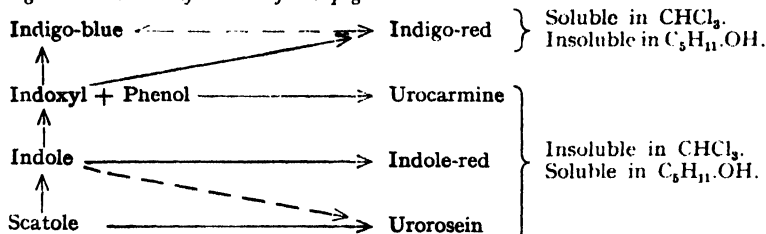
(b) *Cresol indogenides*.

The list might be extended to include the indogenides of the rarer urinary phenols, catechol and resorcinol, which are not volatile in steam.

2. *Indole reds*. Produced by the autoxidation of indole. Only likely to be formed when indole occurs free in urine, a condition so rare as to be improbable.
  - (a) *Indole red* [Oddo (*Gazz. Chim. Ital.*, 1916, **46**, 323); Baudisch and Hoschek (*Ber.*, 1916, **49**, 2579)].
  - (b) *Urorubin* [Weiss (*Klin. Wochenschr.*, 1930, **9**, 248)].
3. *Scatole-reds, or uroroseins*. [Porcher and Hervieux (*J. Phys. Path. Gen.*, 1908, **7**, 815)]. Derived directly or indirectly from the biochemical oxidation of scatole or of tryptophan. Sometimes included among the indole-reds. Chromogen precursors cannot be resolved by steam distillation.
  - (a) *Indolepropionic acid* [Stokvis (*Jahrb. Thierchem.*, 1901, **31**, 444)].
  - (b) *Indoleacetic acid*. Believed by Herter (*J. Biol. Chem.*, 1908, **4**, 253) to be the chromogen of the original urorosein of Nencki and Sieber (*J. prakt. Chem.*, 1882, **26**, 333).
  - (c) *Indolealdehyde*. Believed by Ellinger and Flamand (*Z. physiol. Chem.*, 1909, **62**, 276) to be the chief chromogen of the scatole-reds.
  - (d) *Indolecarboxylic acid*. [Maillard (*Dictionnaire de Physiologie*, 1913, Richet, Paris).]

In alcoholic solution the scatole-reds show well-defined absorption spectra.

*Diagrammatic classification of the pigments.*



Solutions of indole do not give a urocarmine reaction with the appropriate phenols unless the mixture is strongly oxidised (*e.g.* with potassium chlorate), when colours are obtained identical with the corresponding indoxyl colours; this is probably due to the oxidation of indole to indoxyl. Hence, with the use of 3 per cent. hydrogen peroxide and 1 per cent. potassium chlorate as oxidising agents in the cold, it is possible to distinguish between indole and indoxyl, since the former only gives the urocarmine reaction with the chlorate, whereas the latter gives a urocarmine with both oxidising agents. When positive, the urocarmine reaction in urine indicates the presence in the urine of indoxyl and phenols unsubstituted in the *para*-position, and hence constitutes a test for phenoluria. P. H. P.

**Fastness of Dyes to Perspiration.** C. C. N. Vass. (*J. Soc. Dyers and Col.*, 1931, **47**, 9-10).—Reports of the investigation initiated in June, 1928, by the Fastness to Perspiration Sub-Committee of the Fastness Committee of the Society of Dyers and Colourists, under the direction of Prof. B. A. McSwiney, have been published in the *J. Soc. Dyers and Col.*, 1929, **45**, 217; 1930, **46**, 190. Perspiration was collected from a wide range of normal, coloured, obese, and rheumatic male and female subjects, and, with one exception, freshly shed samples were acid, becoming alkaline on standing. The urea nitrogen was always greater than the ammonia nitrogen, but diminished on standing with total disintegration of the urea, and the salt concentration varied from 2.65 grms. per 1000 c.c. to 5.01 grms.

for normal male subjects. The average concentrations of the constituents deduced for each type, which were all very similar, conformed very closely to those of individual analyses. In every case the reducing power of freshly-shed normal female perspiration was greater than that of male, particularly so in the case of stout females. The changes observed on standing are shown to be due to the action of bacteria normally present on the skin. The data established concerning the reactions and constituents of perspiration are being used to formulate perspiration-test liquors.

D. G. H.

**Application of the Seed of *Soya hispida* Deprived of Uricase. Qualitative and Quantitative Analysis.** R. Fosse, A. Brunel, P. de Graeve, P. E. Thomas and J. Sarazin. (*Compt. rend.*, 1930, 191, 1388-1390.)—The authors' method for the selective destruction by heat of uricase in the presence of allantoinase and urease (*id.*, 1930, 191, 1153) is applied to the detection of allantoin, a solution containing 10 mgrms. per litre of which is warmed at 40° C. for 30 minutes with 1 per cent. of active uricase-free soya and chloroform. The mixture is filtered, the filtrate adjusted to 0.1 N acidity by addition of N hydrochloric acid, and heated at 100° C. for 1 minute. Addition of 4 drops per c.c. of 1 per cent. phenylhydrazine hydrochloride to the warm solution followed, after cooling, by 2 drops per c.c. of 5 per cent. potassium ferricyanide solution and finally by 2.5 c.c. per c.c. of concentrated hydrochloric acid, produces a red coloration. With mammalian blood the serum is allowed to ferment overnight at 40° C. with the enzyme, and the proteins coagulated with 20 per cent. of solid trichloroacetic acid. The method, which depends on the conversion of allantoin into allantoic acid, which is then decomposed by the mineral acid into glyoxylic acid and urea, may be used in the presence of uric acid (cf. *id.*, 1929, 188, 1418) if the uricase (which produces allantoic acid from uric acid) is destroyed. The maximum errors obtained were  $\pm 0.9$  per cent. In the case of dog's urine, daily determinations of the total nitrogen (micro-Kjeldahl) and of the urea (xanthidrol) showed that whilst the allantoin content varied between 0.6 and 3.3 grms. per litre, the ratios of allantoin nitrogen to 100 parts of total nitrogen, and of allantoin to 100 parts of urea, were constant (1 to 1.2). The production of allantoic acid by untreated soya from potassium urate is completely inhibited by  $10^{-5}$  parts of potassium cyanide. Allantoin in uric acid should, however, be determined in the presence of 0.6 to 0.8 gm. per litre of cyanide if the untreated soya seed is used, since the limiting inhibiting quantity of cyanide is raised in the presence of allantoin. Below this quantity a portion of the uric acid is fermented, and above it the activity of the allantoinase is inhibited.

J. G.

**Method for Determination of Enzyme Yield in Fungus Cultures.** Z. I. Kertesz. (*J. Biol. Chem.*, 1931, 90, 15-23.)—Fernbach (*Ann. Inst. Pasteur*, 1890, 4, 1, 641) proposed a method and a unit for the determination of sucrase. At that time the method was regarded as of great importance, but later work showed it to have serious defects. The essential mistake was that the enzyme was not determined in the mould directly, but in a solution made up from the

killed cells, and this result was set down as the enzyme content of the mould. The great importance of Fernbach's idea is generally conceded, but his method is no longer used for the determination of the enzyme content of a plant or of an enzyme preparation. A new method is now presented by the author for the determination of the enzyme yield of mould cultures and for obtaining comparable enzyme determinations on solid and liquid materials. As an example, the method is applied to the sucrase of *Penicillium glaucum*, and data are presented to show the significance and suitability of the method. The total enzyme content of the mycelium of a mould culture may be expressed by the formula

$$E_1 = \frac{k \times \text{grms. of sucrose}}{\text{dry matter}} \times (\text{total dry matter yield of the culture in grms.})$$

in which  $k$  is the monomolecular reaction constant, sucrose is the total sucrose content of the reaction mixture in grms., and dry matter the amount of dry mycelium in grms. which supplied the enzyme used. The total sucrase content of the medium can be expressed in the same way, but in this case the calculation is made on the basis of volume rather than weights.

$$E_2 = \frac{k \times \text{grms. of sucrose}}{\text{c.c. of medium used}} \times (\text{volume of entire culture medium in c.c.}).$$

By the addition of these two values ( $E_1 + E_2$ ) a number is obtained which indicates the sucrase, or other enzyme, yield of the whole culture. In duplicate cultures growing on identical media, on the 5th day the  $E_1$  is practically the same, in spite of the fact that the enzyme activity ( $I_{f1}$ ) and dry matter yield vary greatly. With complete salt nourishment the  $E_1$  increases as the sucrose content of the medium is increased. In the absence of one important nutrient element (K,P,Mg), and increasing sucrose supply, the value of  $E_1$  is roughly constant. In *Penicillium glaucum* cultures growing normally, the value of  $E_1 + E_2$  reaches a maximum in the first few days; afterwards it decreases rapidly. With the use of the  $E$  values it is possible to study the sucrase yield of mould cultures, especially the study of the distribution of the enzyme between mycelium and medium, since the values obtained are directly comparable. The proposed method is applicable to the determination of other enzymes in fungal or bacterial cultures, since the same principles apply to the determinations of other enzymes. P. H. P.

**Determination of Peroxydase Activity.** J. D. Guthrie. (*J. Amer. Chem. Soc.*, 1931, 53, 242-244.)—A mixture of  $\alpha$ -naphthol and  $p$ -phenylenediamine is used for the determination of oxydase activity, owing to its oxidation to an indophenol by atmospheric oxygen in presence of the enzyme, but in nearly neutral media is too sensitive for peroxydase. In more acid solutions, in which, moreover, catalase is inactive, the reaction is, however, sufficiently slow to be followed quantitatively, and the procedure involved has now been standardised so as to allow of the comparison of determinations made at different times. The substrate is made by preparing a citrate buffer of pH 4.5 by dissolving 21 grms. of crystallised citric acid in 170 c.c. of  $N$  sodium hydroxide solution and diluting to one litre.

To 200 c.c. of this solution are added 200 c.c. of water, 1 grm. of *p*-phenylenediamine hydrochloride, and 20 c.c. of a 4 per cent. solution of  $\alpha$ -naphthol in 50 per cent. alcohol, the liquid being then filtered. Of this freshly-prepared substrate, 25 c.c. portions are placed in unlippered centrifuge tubes of about 80 c.c. capacity, these being left for a time in a water-bath at 25° C. to acquire this temperature, and then treated with 0.5–2.0 c.c. of the juices or extracts containing the enzyme. The reaction is started by adding 5 c.c. of 0.05 *N* hydrogen peroxide solution and stopped, after the lapse of 10 minutes, by adding 5 c.c. of 0.1 per cent. aqueous potassium cyanide solution. To each tube 25 c.c. of toluene are added, the well-corked tubes being shaken and centrifuged. The clean toluene layers containing the indophenol are compared colorimetrically with the standard. A blank test on the reagents is advisable.

The standard is prepared by adding 100 c.c. of 2 *N* hydrogen peroxide and 2 c.c. of 10 per cent. aqueous ferric chloride ( $\text{FeCl}_3, 6\text{H}_2\text{O}$ ) solution to 800 c.c. of the substrate, the indophenol being filtered off the next day and recrystallised from a 1:1-mixture of absolute alcohol and toluene; 0.05 grm. of this preparation is dissolved in 50 c.c. of the warm mixed solvent, toluene being added to make the total volume one litre. This solution keeps well. A solution of 2 grms. of iodine in a litre of chloroform is also satisfactory as a standard. T. H. P.

#### **Fading of Tropaeolin 00 in the Titration of Organic Acids in Urine.**

**K. L. McCluskey.** (*J. Biol. Chem.*, 1931, **90**, 197–201.)—In a study of urine acidity in tuberculosis the method of Van Slyke and Palmer (*J. Biol. Chem.*, 1920, **41**, 567; *ANALYST*, 1920, **45**, 229) was used for the determination of the organic acids. This method is based on the fact that at *pH* 8 the organic acids in the urine are present as salts, and that the addition of nearly a full molecule of hydrochloric acid for each molecule of the salt of the weak acid is necessary to free the weak acid. This change is practically complete at *pH* 2.7, and the indicator which has been found to be satisfactory, with few exceptions, is tropaeolin 00. Occasionally fading of the indicator occurs, especially near the end-point (2.7). The ingestion of drugs by the subjects has been investigated, but has not been found to be the cause of the fading. It became imperative to find either a more suitable indicator, or a method of removing the interfering substance. As the result of observations a method was found by which the interfering substance could be removed by bubbling air for half an hour through the urine, to which concentrated hydrochloric acid had been added in the proportion of 0.5 c.c. to 100 c.c. of urine. This brings the *pH* of most urine to the neighbourhood of 2.7. It is shown that the aeration of urine in the presence of hydrochloric acid (*a*) will remove an easily oxidisable substance found to react with tropaeolin 00; (*b*) will remove carbonates, a necessity when present in quantities greater than 0.5 per cent.; (*c*) may give values on an average 5 per cent. lower than does untreated urine. This difference is not due to loss of volatile acids, to incomplete removal of the carbonates, to changes in the uric acid, creatinine, and creatine, or to the salt effect upon the indicator, but may be due to pigment content. The urinary

substance found to react with tropaeolin 00 is an easily oxidisable substance, and may be an unoxidised sulphur compound arising from the destruction of tissue protein, and is probably present in other diseases besides tuberculosis. Upon repeated examinations this substance has been found to occur at intervals in 30 to 40 per cent. of the patients in very advanced phthisis.

P. H. P.

**Sterol Content and Antirachitic Activatibility of Mould Mycelia.** L. M. Pruess, W. H. Peterson, H. Steenbock and E. B. Fred. (*J. Biol. Chem.*, 1931, 90, 369-384.)—A brief review of the literature is given which shows that very little is known of the number and kind of sterols found in moulds, and the work that has been done on their identification is more or less conflicting. A study is reported of a large number of moulds which were tested with respect to (1) their ability to grow on a synthetic culture medium, and (2) their antirachitic activatibility. In a preliminary survey, 55 moulds were used to inoculate a synthetic, inorganic medium containing 4 per cent. of glucose as the source of carbon. Of these moulds, twenty-three showed good growth in flasks. The yields of dry pad varied from 7.3 to 35.0 per cent. of the glucose consumed. Eleven of the twenty-three moulds, representing the principal species, were grown in mass cultures in large tin pans. The weight of dry pad ranged from 9.5 to 25.6 per cent. of the glucose utilised. Different strains of the same species also differed in their utilisation of glucose. Increasing the glucose content of the medium to 10 or 20 per cent., in most cases, more than doubled the yield of dry pad. When the inorganic medium was supplemented with an aqueous extract of malt sprouts or fresh yeast, the mycelial growth of one of the strains studied, *Aspergillus oryzae*, Culture 2, was increased from 20 to 50 per cent. Increasing the period of incubation of this strain from 10 to 50 days caused a 56 per cent. decrease in the weight of pad. The autoclaved, dried, and finely-ground pads of 12 of the 23 moulds showing good growth were irradiated with ultra-violet light and given to rachitic rats to test their antirachitic potency. Five kinds of mushrooms, gathered in the open, were similarly tested for their antirachitic activatibility. Eight of the moulds and three of the mushrooms brought about distinct healing of rickets, even when given at the dosage of 10 mgrms. of irradiated material per rat over a period of 7 days. The total alcohol-soluble sterol in eleven moulds and four mushrooms (determined by the gravimetric digitonide method) varied from about 0.1 to 1.0 per cent. of the dry weight of the fungus material. The amount of sterol left unextracted by the alcohol ranged from 0.01 to 0.40 per cent. of the dry pad. In most cases, 90 per cent. or more of the alcohol-soluble sterol was present in the free state. The sterol contents of the moulds varied with the species, and also with different strains of the same species. Increase in the glucose content of the medium caused a decrease in the percentage of sterol. Supplementing the inorganic medium with organic nutrients (aqueous extract of malt rootlets or fresh yeast) produced no appreciable change in the sterol content. Lengthening the period of incubation increased the percentage of sterol; the weight of pad, however, decreased so that the quantity of sterol per unit of apparatus remained about the same.

P. H. P.

**Study of the Antimony Trichloride Colour Reaction for Vitamin A.** E. R. Norris and A. E. Church. (*J. Biol. Chem.*, 1930, 89, 589.)—Several workers have shown that there is a falling off in weight-gain in rats fed for 3 or 4 weeks on a diet containing cod-liver oil, and that this falling off is not arrested by increasing the amount of oil. It is here demonstrated that this phenomenon is due to the vitamin A being destroyed by the oil in which it has been dissolved and stored during the course of the feeding experiment, rather than to the presence of some harmful physiologically active constituent of the diet, such as *iso*-amylamine or choline. A test was carried out on the unsaponifiable, cholesterol-free fraction of cod-liver oil, to ascertain the effect on it of the antimony trichloride colour reaction. Portions of the vitamin fraction were dissolved in chloroform, olive oil, arachis oil and in coconut oil, colour readings being taken before and after 6 weeks' storage in the dark and cold and plotted against concentration. A study of the dilution curves shows that the chromogenic substance was destroyed in the arachis oil, destroyed to the extent of 30 per cent. in the olive oil, and not destroyed in the coconut oil or in the chloroform. Some samples of coconut oil, however, have a considerable destructive action. The destructive action of olive oil can be partly, and of arachis oil completely, overcome by adding 0.002 to 0.05 per cent. of hydroquinone. The lack of weight-gain referred to can be stopped and weight-gain resumed by adding to the vitamin A diet the vitamin B complex of certain yeasts. R. F. I.

**Study of the Effect of Nitrous Acid upon Components of the Vitamin B Complex.** H. C. Sherman and M. L. Whitsitt. (*J. Biol. Chem.*, 1931, 90, 153–160.)—Experiments are reported which were made with the purpose of throwing further light upon the differentiation of vitamins  $B(B_1)$  and  $G(B_2)$  by means of strictly parallel measurements of the effects of nitrous acid upon these two vitamin values or potencies in the same solution. The "protein-free milk" preparation of Osborne and Mendel, containing both of these vitamins, was chosen for the work. The investigation extends that of previous workers, first, by the use of different nitrous acid treatments (including a more drastic form), and second, by the strictly parallel study of the two vitamin potencies by rat growth methods in the same material before and after treatment. The authors have sought to interpret the experimental data in the light of the present evidence that more than two components of the vitamin B complex may be involved. A preliminary experiment indicated that neither the vitamin B nor vitamin G concentration of the protein-free milk solution was measurably diminished by standing 14 hours at room temperature acidulated with hydrochloric acid (1:1 concentration) as necessary in the more drastic nitrous acid treatment. The results show that treatment by the aspiration method had little if any influence upon vitamin  $B(B_1)$ , whereas the drastic treatment with nitrous acid, generated very abundantly directly in the vitamin solution, resulted in a large diminution of the vitamin  $B(B_1)$  potency, as shown both by the weight curves of the experimental animals and the development of polyneuritic symptoms. Less drastic direct treatment yielded an intermediate result. It is suggested that vitamin B may be a nitrogenous base, but of



such structure as to be more resistant to nitrous acid than are the typical primary amines. There was no destruction of vitamin  $G(B_9)$  under treatment by the aspiration method; with the drastic method, subsequent feeding tests indicated a partial destruction of vitamin  $G$  when the data were taken for an 8-week, but not when taken for a 4-week, experimental feeding period. It is probable that vitamin  $G(G_1, B_9)$  is itself stable toward nitrous acid, but that certain indications of loss of vitamin  $G$  potency show more or less destruction of one of the newer and not yet clearly defined factors, which also are now being found to be essential to the growth of rats. Whatever the component of the vitamin  $G$  complex which is affected by the nitrous acid treatment, there are indications that the reaction involved may be one of oxidation rather than deamination in this case. From the results it appears that vitamin  $B$  behaves *in vitro* more like a nitrogenous base, and vitamin  $G$  more like a neutral organic substance. P. H. P.

**Colour Reaction of Japanese Acid Clay with Carotene.** K. Kobayashi, K. Yamamoto and J. Abe. (*Chem. News*, 1931, 142, 66.)—Japanese acid clays dried for two hours at  $150^\circ$  acquire an intense activity in the colour test for carotene in a medium of benzene; with alcohol or ether, the results were unsatisfactory. Japanese acid clay gives the same vivid bluish-green colour with carotene, liver oil, and vitamin  $A$  as is produced by phosphoric acid and the anhydrous chlorides of aluminium and zinc. (Cf. *ANALYST*, 1927, 52, 553; 1929, 54, 562.) R. F. I.

## Toxicological and Forensic.

**Dangerous Properties of Ethylene Chlorhydrin.** J. D. Pratt. (*Nature*, 1930, 126, 995.)—Attention is drawn to fatal cases (*Zentr. Gewerbehygiene*, 1927, 4, 712; *Ann. Rept. of Chief Inspector of Factories and Workshops*, 1930, 95) resulting from inhalation of vapours of ethylene chlorhydrin, which act as a metabolic poison having a specially severe effect on the nervous system and producing muscular weakness, inertness, refusal of food, sleepiness, and finally death by paralysis of respiration. These toxic effects have apparently hitherto been unsuspected (cf. Denny, *J. Ind. Eng. Chem.*, 1928, 20, 578). J. G.

**Detection of Sperma in Forensic Cases.** J. Peltzer. (*Chem. Ztg.*, 1931, 55, 70.)—The following simple and rapid method gives trustworthy results: The suspected spots on the clothing are dabbed in various places with 3 per cent. hydrogen peroxide solution by means of a glass rod. The presence of sperma gives rise to a copious froth, which is removed by placing a microscope slide on it. Secretion which has penetrated the fabric may be recovered by pressing the treated spots with tweezers and scratching the expressed froth with a platinum loop, from which it is transferred to the slide. Some of the preparations are then treated with a platinum loopful of 2 per cent. aqueous eosin solution. Others are fixed by a short exposure to a flame and then covered with iodine in potassium iodide solution; the presence of sperma is indicated by the formation of long, pointed, chocolate-brown crystals, which disappear after a few minutes, but

reappear on addition of fresh iodine in iodide solution. If no crystals form, microscopic examination of the stained and unstained preparations will fail to reveal spermatozoa.

T. H. P.

## Bacteriological.

**New Coli Test.** A. Van Raalte. (*Chem. Weekblad*, 1930, 27, 663.)—In examining milk, 1 c.c. is added to 9 c.c. of a medium containing 10 grms. each of peptone and of sodium chloride and 250 mgrms. of sodium carbonate per litre. Successive dilutions are then made from 1 c.c. of mixture and 9 c.c. of medium so as to give a range containing 1 part of milk in 10, 100, etc., to 100,000. These are tested for indole after 24 hours at 37° C. No reaction should be obtained for a dilution of 1 in 10,000 of ordinary trade milk, or 1 in 100 of "model" or pasteurised milk. Carelessly prepared milk may give a positive reaction in dilutions of 1 in 100,000. The 1 in 10 dilution usually gives negative results owing to coagulation.

J. G.

**The Aspergilli and their Relation to Decay in Apples.** G. A. Huber. (*J. Agric. Res.*, 1930, 41, 801–817.)—The Jonathan apples, the fungus flora of which was to be examined, were wrapped in sterile wraps immediately on picking, sorted, packed, shipped, and kept in cold store until they were washed in the culture room with stiff stencil brushes for 5 minutes, in a sterile chamber containing 100 c.c. of sterile water. Plates were then made in triplicate with 0.125, 0.25, 0.5, and 1 c.c. from the thoroughly shaken wash waters, potato agar containing 2 per cent. dextrose being used as medium, and 1 drop of 25 per cent. lactic acid being added to each tube of medium before pouring, to inhibit bacterial growth. Between 1000 and 271,000 fungus colonies were yielded by each apple, representing altogether 11 forms of *Aspergillus* isolated from the surface of normal apples. Seven of these forms caused decay when inoculated into normal apples under storage conditions at temperatures of 18° to 22° C., but none of them caused decay at cold storage conditions (0°C.). The forms of decay varied from firm, moist rots to dry, leathery rots, whilst *Aspergillus niger* caused a very soft watery rot.

D. G. H.

## Organic Analysis

**Influence of Acid Chlorides and of Pyrrole on the Colour Test for Reactive Organo-metallic Compounds.** H. Gilman and L. L. Heck. (*J. Amer. Chem. Soc.*, 1930, 52, 4949–4954.)—It has been found that some acid chlorides interfere, by giving disturbing colours, with the sensitive colour test for reactive organo-metallic compounds, as, for example, Grignard reagents, described by Gilman and his collaborators (e.g. *J. Amer. Chem. Soc.*, 1925, 47, 2002; *id.*, 1930, 52, 1604), which consists in adding a small volume of the organo-metallic mixture to be tested to Michler's ketone in benzene, hydrolysing with water, and then developing the colour with a glacial acetic acid solution of iodine. Such interference is not general, but has been noted with benzoyl chloride and bromide, phosgene, oxalyl chloride, thionyl chloride and phosphorus pentachloride. Certain amines also interfere; in particular, pyrrole.

S. G. C.

**Gravimetric Determination of Formaldehyde and Urotropine.** M. V. Ionescu and C. Bodea. (*Bull. Soc. Chim.*, 1930, IV, 47, 1408-1419).—In absence of other aldehydes, formaldehyde may be determined by means of its reaction with dimethyldihydroresorcinol (methone) (ANALYST, 1928, 53, 507). The cold, aqueous, neutral solution of not more than 0.3 grm. of formaldehyde is treated with excess of the clear methone solution (0.5-1 per cent.), the liquid being either left for 6 hours at the ordinary temperature, with occasional stirring, or boiled for 10 minutes, then allowed to cool, and allowed to stand for 30 minutes. The crystalline precipitate formed ( $C_{17}H_{24}O_4$ ) is collected in a Gooch crucible, well washed with cold water and dried at 110-115° C. Multiplication of the weight of the precipitate by 0.10274 gives the amount of formaldehyde.

Urotropine may be determined similarly, the precipitation being carried out in the boiling solution, which is left to cool before filtration. The weight of the precipitate is converted to that of the urotropine by multiplying by 0.07993. The difference between the velocities of reaction of formaldehyde and urotropine with the methone is not sufficiently great to allow of the determination of both compounds when present together.

T. H. P.

**Oil of Amber (*Oleum Succini*).** T. T. Cocking. (*Perf. and Ess. Oil Record*, 1930, 21, 477-478).—Genuine oil of amber, which is obtained by destructive distillation of genuine amber, is usually substituted in this country by "Oil of Amber" (*Oleum Succini Rect.*), obtained similarly from other resins. The physical properties of 3 samples of the latter of different origins are tabulated, and the resulting variations obtained indicate that the limits of the B.P. Codex are too narrow. The following more complete characters are suggested:—Sp. gr. 0.850 to 0.875,  $[\alpha]$   $-12^\circ$  to  $+12^\circ$ ,  $n$  ( $20^\circ$  C.) 1.465 to 1.482, solubility in 90 per cent. alcohol 1 in 5 to 6 volumes. Corresponding values and other characteristics are also tabulated for genuine oil prepared by the author (by distillation below  $300^\circ$  C., separation of the acid layer and redistillation in a vacuum), and by Messrs. Schimmel (1903), and for commercial oil prepared by the author from colophony. The author's values for the genuine oil are: Sp. gr. 0.935;  $[\alpha]$   $+20^\circ$ ;  $n_D^{20}$ , 1.5155; acid value, 13.0; ester value, 2.0; saponification value, 15.0; boiling below  $150^\circ$  C. 0, at 150 to  $200^\circ$  C. 15 per cent.; 200 to  $250^\circ$  C., 15 per cent.; 250 to  $300^\circ$  C., 43 per cent. The lower fractions of the oil from colophony had a terebenthic odour, but the higher fractions had the true odour of *Oleum Succini Rect.* The genuine oil was golden-yellow, but darkened on storage.

J. G.

**Anti-oxidants and the Autoxidation of Fats.** H. A. Mattill. (*J. Biol. Chem.*, 1931, 90, 141-151).—The control of autoxidation reactions, by means of anti-oxidants and pro-oxidants (promoters), is increasingly useful in industry. To secure information on the chemical nature of the anti-oxygenic substances that are found in natural oils and that prevent the autoxidative destruction of fat-soluble vitamins, a series of hydroxy aromatic compounds was tested for their capacity, when used in a quantity of 0.02 per cent. of the fat mixtures, to prolong the thermal oxidation induction period of a standard mixture of lard and cod-liver

oil. The observations indicate that the anti-oxygenic capacity of phenols resides in two hydroxyl groups in the ortho- or para-configuration; when the groups are in the meta position the compound is inactive. The hydroxyls are ineffective unless attached directly to the ring; the fully hydroxylated inositol is inactive. In the naphthols one hydroxyl group is sufficient, and, in keeping with its accepted behaviour,  $\alpha$ -naphthol has the character of an ortho compound, and is much more effective (more than 10 times), as an anti-oxidant, than  $\beta$ -naphthol. Quinone is effective, and  $\beta$ -naphthoquinone is even more so, but the  $\alpha$  form of the latter is entirely inactive. The relation of these facts to the more recent theories of the electronic structure of the benzene ring and autoxidation is briefly discussed, and it is suggested that in the preparation and manipulation of easily autoxidisable substances, the presence of traces of anti-oxidant will prevent undesirable oxidative changes. A number of sterols of animal origin and sitosterol from wheat, maize and lettuce were all inactive. This was to be expected, since there is only one hydroxyl, although the presence of some other sufficiently positive group might serve in its place. The existence of pro- and anti-oxygenic substances among the non-saponifiable constituents of natural fats and oils suggests that some of these may be concerned with the physiological action of the fat-soluble vitamins, and methods for their segregation from the sterols are under investigation.

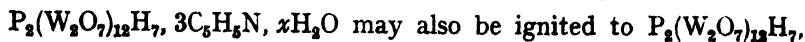
P. H. P.

**Identification of Phenols.** C. F. Koelsch. (*J. Amer. Chem. Soc.*, 1931, **53**, 304–305.)—Good yields of the sodium salts of aryloxyacetic acids are obtained by treating phenols, dissolved in aqueous sodium hydroxide, with chloroacetic acid. The acids themselves are crystalline solids, easily purified by crystallisation, and sufficient is obtained from 1 grm. of a phenol to allow of the determination of the equivalent and melting point. A mixture of 1 grm. of the phenol and 3.5 c.c. of 33 per cent. sodium hydroxide solution is treated with 2.5 c.c. of 50 per cent. chloroacetic acid solution, a little water being added when necessary to dissolve the sodium salt of the phenol. The test-tube is stoppered loosely and heated for one hour in a gently-boiling water-bath. The solution is then cooled, diluted, acidified to Congo red with a mineral acid, and extracted once with ether, the ethereal extract being washed once with a little water and the aryloxyacetic acid removed by washing with dilute sodium carbonate solution. Acidification of this extract gives the free acid, which is recrystallised from water. The uncorrected melting points of the acids thus obtained from a number of phenols are as follows:—Phenol, 98–99° C.; *o*-, *m*-, and *p*-cresols, 151–152° C., 102–103° C., and 134–136° C., respectively; *o*-, *m*-, and *p*-chlorophenols, 143–145° C., 108–110° C., and 155–156.5° C.; *o*-, *m*-, and *p*-bromophenols, 141–143° C., 107–108.5° C., and 157° C.; *o*-, *m*-, and *p*-iodophenols, 134–135° C., 114–115.5° C., and 154–156° C.; *o*-, *m*-, and *p*-methoxyphenols, 116–116.5° C., 111–113° C., and 110–112° C.; thymol, 148–149° C.; carvacrol, 150–151° C.;  $\alpha$ -naphthol, 191–192° C.; and  $\beta$ -naphthol, 153–154.5° C. Certain of these results differ somewhat from those recorded in the literature, and in other cases no previous determinations are recorded.

T. H. P.

**Detection of  $\beta$ -Naphthol.** G. De Haas. (*Pharm. Weekblad*, 1931, 68, 29-32.)—A modification of Autenrieth's test for thymol ("*Auffindung der Gifte*," p. 55) is described, in which 1 c.c. of a dilute aqueous solution of  $\beta$ -naphthol containing a little alcohol is mixed with 5 c.c. of glacial acetic acid, and a lower layer of 5 c.c. of concentrated sulphuric acid added. A yellow-green colour is produced as a ring at the liquid-junction, and, on shaking, spreads throughout the mixture as a fluorescence. Thymol, cresols, phenol and guaiacol give violet, brown, violet and red colours, respectively, whilst  $\alpha$ -naphthol gives a ring of indefinite shade and no fluorescence. The test has a maximum sensitiveness of 1 in 100,000 (Millon's reagent and bromine 1 in 10,000, ferric chloride 1 in 1,000), and is due to a sulphonation product of  $\beta$ -naphthol which is not extractable by ether, chloroform or petroleum spirit. J. G.

**Detection and Determination of Pyridine.** S. B. Tallantyre. (*J. Soc. Chem. Ind.*, 1930, 49, 466-468T.)—"Pyridine bases" from tar distillates are usually assumed to include pyridine, picolines, lutidines and collidines (b.pt.  $90^\circ$  to  $160^\circ$  C.), and are completely soluble in water, whilst "heavy bases" are immiscible with water (b.pt.  $160$  to  $250^\circ$  C.), and include lepidines, quinolines, etc. **Distillation.**—For the separation of the former by distillation, especially in the presence of ammonia, the pH value should be adjusted to about 10 by cautious addition of sodium hydroxide solution till a very faint pink is obtained with phenolphthalein. Ladd's method (ANALYST, 1919, 44, 299) is recommended for titration of the distillate, and the factor 0.75 converts the bases, calculated as pyridine, into "pyridine bases." **Extraction.**—About 80 grms. of sodium chloride are dissolved in 250 c.c. of (e.g. tar- or gas-) liquor, and the solution extracted with 150, 100 and 100 c.c. portions of benzene, the combined extracts being washed with concentrated salt solution and dried over sodium sulphate. The bases are then extracted in 25 c.c. of 0.5 N sulphuric acid, followed by two 15 c.c. portions of water, and the combined extracts titrated with 0.5 N sodium hydroxide solution (cf. Ladd, *supra*). **Precipitation.**—Mercuric chloride (5 per cent.) has a sensitiveness of 1 in 1,000, and iodine (Harvey and Sparks, *id.*, 1918, 43, 146) 1 in 200,000, though the latter is recommended only when the solution is quite clear and when a gravimetric result is not required. Phosphotungstic acid (5 per cent.) should be used in slight excess in the presence of about 1 per cent. of mineral acids and in the absence of ammonia and of large quantities of salts. It then serves as a nephelometric method having a sensitiveness of 1 in 50,000. The precipitate,



whence the factor 0.041 gives the amount of pyridine. Silicotungstic acid (5 per cent.) is less sensitive to pyridine bases (1 in 8,000), and more sensitive to heavy bases (1 in 50,000), and is affected less by ammonia. It is used in a faintly acid medium, and the factor 0.12 is used for conversion of the weight of residue after ignition ( $SiO_2, 12WO_3$ ). Spacu's pyridine test for copper (ANALYST, 1925, 50, 580 *et seq.*) may also be used, 25 c.c. of a 0.02 per cent. solution of pyridine being mixed with 2.5 c.c. of 5 per cent. potassium thiocyanate solution, 5 drops of

5 per cent. copper sulphate solution and 5 c.c. of chloroform, when the chloroform is coloured green. Analogues of pyridine do not react, but aniline and piperidine give similar compounds. A specific test for pyridine in aqueous solution, in the presence of ammonia, quinoline, nicotine or pyrrole, is the yellow colour, turning to a brown precipitate, produced on shaking 50 c.c. of sample (1 of pyridine in 350,000) with 1 drop of aniline and 1 c.c. of a fresh mixture of 3 volumes of bromine water and 1 of 5 per cent. potassium cyanide solution. The colour may be matched against that produced from a standard, and the test has been applied to ammonium sulphate and methylated spirits. In the latter case 5 c.c. are acidified with sulphuric acid, the alcohol removed on the water-bath, the residue extracted with 25 c.c. of water, and the filtrate neutralised with sodium hydroxide and tested. J. G.

**Determinations of Piperidine in a Mixture of Pyridine and Higher Homologues.** A. Travers and Franquin. (*Compt. rend.*, 1930, 191, 1340–1343.)—The neutralisation-curves of these bases were obtained by addition of various quantities of each base to 25 c.c. of 0.01 or 0.02 *N* sulphuric acid, and determination of the corresponding *pH* values by means of the quinhydrone or hydrogen electrode. On account of the volatility of pyridine and  $\alpha$ -picoline, measurements were made at 0° C. against a saturated calomel electrode at 15° C. It is then calculated that, assuming the *pH* of the sulphuric acid to be the same at 0° C. as at 15° C., the *pH* of the mixture at the various stages of neutralisation is given by  $(E - 260)/54.1$ , where *E* is the corresponding measured potential-difference in millivolts. The ionisation constants derived from the curves are, pyridine  $0.42 \times 10^{-9}$  (0° C.),  $\alpha$ -picoline  $1.7 \times 10^{-8}$  (0° C.),  $\alpha\alpha'$ -lutidine  $3.3 \times 10^{-7}$  (15° C.), and piperidine  $1.10 \times 10^{-3}$  (15° C.). The  $\alpha\alpha'$ -lutidine and  $\alpha$ -picoline, and particularly piperidine, give sharp end-points, and piperidine may thus be titrated in admixture with the other bases after distillation with barium hydroxide to destroy any piperidine bicarbonate. A curve is also shown for 10 c.c. of 0.02 *N* piperidine mixed with 5 c.c. of each of the other bases in 0.02 *N* solution in each case.

J. G.

## Inorganic Analysis.

**Rapid Gravimetric Determination of certain Elements.** J. Dick. (*Z. anal. Chem.*, 1930, 82, 401–415.)—The procedure, consisting in washing a precipitate with alcohol, then ether, and weighing it after drying *in vacuo* (ANALYST, 1927, 52, 494, 660; 1928, 53, 508, 509; 1929, 54, 618), has been extended to zinc, manganese, cobalt, and cadmium ammonium phosphates, elementary bismuth, selenium, and tellurium, and cuprous thiocyanate. W. R. S.

**Interference of Alkaline Earths in the Determination of Lead.** J. Majdel. (*Z. anal. Chem.*, 1931, 83, 36–45.)—The influence of the sulphates of barium, strontium, and calcium on the solution process of lead sulphate in ammonium acetate (after the familiar evaporation of the solution to fumes of sulphuric acid) was submitted to an exhaustive study. It was confirmed that barium strongly interferes, the precipitated  $(\text{Pb}, \text{Ba})\text{SO}_4$  being insoluble in ammonium acetate.

The following losses (in per cent. of lead taken) were observed: Pb:Ba as 10:1, 5.5; 1:1, 46.5; 1:2, 80.5; with 1:10, all the lead remains insoluble. Strontium markedly interferes, but the error is positive, as  $(\text{Pb}, \text{Sr})\text{SO}_4$  is soluble in ammonium acetate. Small amounts of calcium are harmless, whilst, with increasing quantities ( $\text{Pb}:\text{Ca} < 1:2$ ), a positive error in the lead result is obtained. For the accurate separation of lead from the alkaline earths, it is necessary to convert the lead into sulphide.

W. R. S.

**Determination of Small Amounts of Zinc in the Presence of Lead Salts.** M. E. Stas. (*Pharm. Weekblad*, 1931, 68, 93-97.)—In the author's version of Berg's method (*ANALYST*, 1927, 52, 494), 45 c.c. of test solution, 5 c.c. of 10 per cent. acetic acid and 2 grms. of sodium acetate are treated at 60° C. with 3 c.c. (or sufficient excess to produce a canary-yellow colour) of a fresh alcoholic 2 per cent. solution of *o*-hydroxyquinoline. The mixture is heated to boiling, filtered after 24 hours on a 7 cm. paper, and the precipitate washed with 40 c.c. of water. The filter is then placed in the neck of an Erlenmeyer flask, and the precipitate washed through with 2 N hydrochloric acid followed by water, and titrated with 0.05 N potassium bromate solution according to the author's method (*Pharm. Weekblad*, 1930, 67, 1245, and Berg, *loc. cit.*), when 1 c.c. = 0.408 mgrm. Zn. Under these conditions 100 mgrms. or less of lead do not react (*cf.* Marsson and Haase, *ANALYST*, 1929, 54, 494), and the maximum recorded error for 0.1 to 2.5 mgrms. of zinc with 10 to 100 mgrms. of lead is +0.04 mgrm.

J. G.

**Detection and Determination of Nickel in Presence of much Cobalt.** F. Feigl and H. J. Kapulitzas. (*Z. anal. Chem.*, 1930, 82, 417-425.)—The limitations of the dimethylglyoxime reaction, as applied to traces of nickel, are discussed. A new method is described, capable of detecting and determining the trace of nickel invariably present in cobalt salts guaranteed nickel-free. The salt (15 to 25 grms.) is dissolved in a minimum of water, and the solution treated with a saturated one of potassium cyanide until the precipitate has re-dissolved. The cobaltocyanide is oxidised with 3 per cent. hydrogen peroxide, and the solution warmed; if not clear yellow, more peroxide is required. The excess is destroyed by evaporation to one-quarter bulk. A slight precipitate, insoluble in excess of cyanide, is filtered off. The filtrate (200 to 300 c.c.) is treated with solid dimethylglyoxime, warmed to about 60° C., and 40 per cent. formaldehyde added until the liquid smells of it:  $\text{K}_2\text{Ni}(\text{CN})_4 + 2\text{HCOH} = \text{Ni}(\text{CN})_2 + 2\text{CN} \cdot \text{CH}_2 \cdot \text{OK}$ . The nickel cyanide reacts with the solid reagent, the cobaltcyanide remains unaffected. After standing 1½ hours, the precipitate is collected, washed, dissolved in dilute hydrochloric acid, the solution filtered, and the nickel precipitated as usual with one per cent. alcoholic dimethylglyoxime. The method is applicable to cobalt salts containing much iron. Cobalt salts absolutely free from nickel may be prepared by the above procedure; the filtrate from the nickel precipitate, containing cobaltcyanide, is evaporated to dryness on a sand-bath, and the residue stirred and heated till charred. It is made into a paste with water and

strong hydrochloric acid, and heated some hours on the water-bath. The liquid is diluted and filtered, and the filtrate reserved. The residue is dried and ignited, mixed with hydrochloric acid, then water, the liquid filtered and added to the first filtrate. From the solution the cobalt is recovered by precipitation with alkali.

W. R. S.

**Determination of Metallic Iron in Ores.** W. Ackermann. (*Chem. Ztg.*, 1931, 55, 30.)—A process has been proposed (Wilner-Merck, *Z. anal. Chem.*, 1902, 41 710; Treadwell, *Lehrbuch*, p. 527) for the determination of metallic iron when in admixture with iron oxides, in which the mixture is treated with mercuric chloride solution, when, it is claimed, the metallic iron reacts, giving ferrous chloride which can be titrated, and the non-metallic portion is unattacked. The present author has tested the applicability of this process to the determination of metallic iron when mixed with various iron compounds with which it is likely to be associated in analytical samples of metallurgical products. Satisfactory results were obtained for metallic iron in synthetic mixtures with ferrous or ferric oxides, red iron ore and magnetite. With certain mixtures, however, *e.g.* containing metallic iron and brown iron ore, low results were obtained, whilst, on the other hand, mixtures containing cementite, ferrophosphorus or iron sulphide gave excessively, and iron pyrites slightly, high results for the metallic iron content.

S. G. C.

**Determination of Carbon in High Sulphur Steels by Direct Combustion.** H. A. Bright and G. E. F. Lundell. (*Bureau of Standards J. Research*, 1930, 5, 943-949.)—The oxides of sulphur, which are formed in the determination of carbon by direct combustion in oxygen, cause high results if they are not removed from the gas stream, and special attention is necessary to secure the complete preferential absorption of these oxides when the sulphur content of steel is greater than 0.10 per cent. These oxides of sulphur have been shown to consist of sulphur trioxide with more or less sulphur dioxide. The efficiency of the absorbents for these oxides commonly used in the analysis of ordinary steels, *e.g.*, granulated zinc, chromic acid in water or concentrated or dilute sulphuric acid, a column of "ironised" asbestos in the exit end of the combustion tube, or substances added to the sample, has been found insufficient for high sulphur steels, particularly in continuous routine work. Lead dioxide heated at 280° C. is fairly good, but it has certain disadvantages in that it requires an additional furnace, and it must be heated at 250° C. until a uniform blank is obtained; moreover, it must be kept out of contact with carbon dioxide of the air when not in use. Tests have been made of a method of absorption due to H. E. Slocum, which consists of a train in which the gases from the combustion tube are passed first over platinised silica gel heated to 440° C., which acts as a catalyst for oxidising sulphur dioxide, and then through a tower containing closely packed ironised asbestos for removing sulphur trioxide. This method is satisfactory, provided that there is sufficient contact surface of platinised silica gel, although no data are available at present as to how long the catalyst will function satisfactorily. The method of absorption of sulphur oxides advocated in the paper employs chromic acid in conjunction



with asbestos as follows:—The exit gases from the combustion tube are first passed through a special absorption tube (of which a diagram is given) containing about 8 to 10 ml. of a 50 per cent. aqueous solution of chromic acid in the right arm and a 9 to 10 cm. column of closely packed asbestos, plain or ironised, in the left arm. All of the sulphur dioxide is removed by the chromic acid. Some, but not all, of the sulphur trioxide is condensed during the passage through the asbestos and the solution. The first tube is followed by another of identical design, which contains sulphuric acid (97 per cent.) in the right arm, and asbestos in the left arm. Thereafter the gases pass through "anhydrone" (anhydrous magnesium perchlorate) to complete the drying and also to condense sulphur trioxide mist which may have escaped absorption in the first two tubes. The carbon dioxide is absorbed in "ascarite" (with anhydrone) contained in a Fleming tube. The paper contains many details of interest to steel chemists.

S. G. C.

**Detection of Cobalt as Caesium Cobaltinitrite.** H. Yagoda and H. M. Partridge. (*J. Amer. Chem. Soc.*, 1930, 52, 4857–4858).—The solubility in water of caesium sodium cobaltinitrite,  $\text{Cs}_2\text{NaCo}(\text{NO}_2)_6$  (0.05 mgrm. per c.c. at 17° C., Rosenbladt, *Ber.*, 1886, 19, 2531), is lower than that of potassium cobaltinitrite, and serves as a more delicate test for cobalt than the latter salt. The mixture of cobalt and nickel sulphides, from the usual procedure, is dissolved in *aqua regia* and evaporated just to dryness. The residue is dissolved in 1 or 2 c.c. of 6*M* acetic acid. To this solution 2 c.c. of 6*M* sodium nitrite and 0.5 c.c. of 0.5*M* caesium nitrate are added. Precipitation of the yellow caesium sodium cobaltinitrite was found to be instantaneous when more than 0.5 mgrm. per c.c. of cobalt was present, but it required 2 minutes to reveal 0.05 mgrm. per c.c. The sensitiveness of the test may be increased by substituting potassium nitrite for sodium nitrite in the method, when, it is claimed, a solution containing 0.01 mgrm. of cobalt per c.c. will yield a yellow precipitate (probably  $\text{Cs}_2\text{KCo}(\text{NO}_2)_6$ ) in about 3 minutes. Considerable quantities of iron, manganese, or nickel do not interfere.

S. G. C.

**Determination of Magnesium with 8-Hydroxyquinoline—Gravimetrically, Volumetrically and Colorimetrically.** A. W. Hough and J. B. Ficklen. (*J. Amer. Chem. Soc.*, 1930, 52, 4752–4755).—The scope of this paper is the application of the already established 8-hydroxyquinoline method to the rapid determination of magnesium in dilute solutions, particularly boiler-feed waters. The solution (50–100 c.c.) is made ammoniacal with 20 c.c. of "ammonia reagent" (not specified), heated to 70° C., and 100 c.c. or more of a solution of 8-hydroxyquinoline (0.05 per cent.) added, the necessary excess of which is shown by the yellow colour of the filtrate. The precipitate is washed, after filtration, with a dilute solution of ammonia, ignited, and weighed as magnesium oxide. Good results were obtained over the range 1–10 mgrms. of magnesium. In the volumetric method, the filter carrying the precipitate is digested with hot *N* sulphuric acid (100 c.c.), and this is titrated (apparently in presence of the filter paper) with potassium permanganate (4.17 grms. per litre) until the pink colour persists for

2 minutes. Fairly good results were obtained with amounts of magnesium within the range 0.1–3.5 mgrms., beyond which the end-point of the titration became obscure; 1 c.c. of the permanganate solution is equivalent to 0.0001 mgrm. of magnesium (on what this is based is not stated). In the colorimetric method, use is made of the yellow colour of the uncombined 8-hydroxyquinoline remaining in the solution after filtration of the magnesium salt. The depth of colour, which varies in the opposite direction to the amount of magnesium in the sample, is compared in a "100 c.c. colorimeter," the 8-hydroxyquinoline reagent being used as the standard; 1 c.c. = 0.0000416 gm. Mg. Fairly good results were obtained over the range 0.5–5.0 mgrms. S. G. C.

**Quantitative Precipitation of Sulphides in Buffered Solutions. I. Cobalt Sulphide.** M. E. Haring and M. Leatherman. (*J. Amer. Chem. Soc.*, 1930, 52, 5135–5141.)—The paper contains the results of a study of the precipitation of cobalt sulphide from pure solutions of cobalt chloride over a range of pH values 6.15–3.10, adjusted by additions of ammonium acetate and acetic acid, and measured by a hydrogen electrode before the admission of hydrogen sulphide. The solution contained in an Erlenmeyer flask was heated to boiling, and hydrogen sulphide was passed through the flask, but not through the solution, for about 5 minutes, after which the exit was closed and the solution allowed to cool under the pressure of the Kipp's apparatus (45 minutes to 1 hour, overnight for low pH values). After filtration, the precipitate was not washed and the cobalt was determined by roasting the precipitate and reducing it in hydrogen at 1000° C.; a reduction temperature of 800° C. was found insufficient to prevent the cobalt being pyrophoric. At the higher pH values precipitation was very rapid, and the precipitate was flocculent and bulky; the speed of precipitation diminished with diminishing pH and the precipitate was compact. Precipitation was complete within 0.5 per cent. down to pH 3.6, but thereafter low results were obtained. The optimum pH value for the precipitation is stated to be 3.93, when the precipitate is compact and granular.

About the same quantities of cobalt (0.2 gm.) as chloride in the same volumes of solution (90 c.c.) were used throughout the work. From a few experiments, the numerical results of which are not communicated, it is stated that large quantities of ammonium salts and a low concentration of cobalt ion appear to diminish somewhat the precision of the method.

**II. Nickel Sulphide.** M. E. Haring and B. B. Westfall. (*Id.*, 5141–5145.)—A similar study was made of the precipitation of nickel sulphide over the pH range 6.81–3.30. The solutions (90 c.c.) containing 0.2 gm. of nickel (as nickel ammonium sulphate), and the requisite ammonium acetate and acetic acid were heated to 90° C., before saturation with hydrogen sulphide, and, just before the end of the precipitation, the temperature was raised to 60° C.; this modification helped to reduce the adsorption of nickel sulphide by the glass. The precipitate was filtered off on a platinum Gooch crucible, and the nickel weighed as nickel oxide after roasting at 1000° C.; reduction in hydrogen, even at 1000° C., was

not practicable, because of the pyrophoric nature of reduced nickel. The precipitation of nickel sulphide was found to be quantitative down to pH 4, and 4.4 is stated to be the optimum pH value for the precipitation, i.e. the lowest value consistent with complete precipitation and a reasonable time for it (about 1 hour).  
S. G. C.

**Detection of Chlorate in Perchlorate.** T. P. Raikowa-Kowatschewa. (*Z. anal. Chem.*, 1930, 82, 415-417.)—The perchlorate solution (1 to 3 c.c.), or 1 gm. of the finely-powdered salt, is shaken with 5 c.c. of fresh hydrogen sulphide water, and left for a short time. If chlorate is present, an opalescence appears after about 15 seconds, and a white opaque solution results after 3 to 4 minutes. Chlorate-free perchlorate yields a perfectly clear solution. Nitrate dissolves to a clear solution which becomes opalescent after about 10 minutes, but not opaque on longer standing.  
W. R. S.

**Direct Nesslerisation of Ammonia in Sea Water.** H. Wattenberg, (*Conseil Perm. Internat. Explor. Mer. Rapp. Reunion*, 53, 108; *Pharm. J.*, 1931, 126, 11).—Ammonia in sea-water may be determined by direct addition of Nessler's reagent to the sample, provided that precipitation of the calcium and magnesium is prevented by previous addition of Rochelle salt. Five c.c. of a 30 per cent. solution of this tartrate are added to 100 c.c. of the sample, the mixture is added to 10 c.c. of 20 per cent. sodium hydroxide, the 2 c.c. of Nessler's reagent then added and the colour compared with that given by standards after standing for 15 to 45 minutes.  
R. F. I.

## Microchemical.

**Micro-chemical "Spot" Tests for some of the Heavy Metals, using Dithizon (Diphenyl-thiocarbazone).** H. Fischer. (*Mikrochem.*, 1930, 8, 319-329.)—A dilute solution of dithizon in carbon tetrachloride or carbon disulphide (1-2 mgrms. per 100 c.c.), when shaken with a dilute, nearly neutral, solution of many of the mono- or di-valent heavy metals, changes in colour. Details of the tests are given for copper, lead, silver, zinc, and cobalt, and the smallest amount recognisable in the presence of various proportions of other metals is given in each case. The tests may be carried out on a porcelain "spot" plate, in micro-test tubes, or by means of filter paper impregnated with the solution of the reagent.

**Copper.**—The test for copper, in which the green colour of the reagent is changed to brown, is carried out either in neutral solution, when in a drop of 0.05 c.c. the smallest amount recognisable is 0.03γ of copper, or in the presence of 2 per cent. ammonia, when 0.02γ of copper can be detected, or in the presence of 2 per cent. ammonia and 2.5 per cent. ammonium chloride, when 0.03γ of copper can be detected. The copper is recognisable in the presence of more than 30,000 times the quantity of lead, zinc, cadmium or nickel. The presence of 10 per cent. of acetic acid makes the test about 500 times less sensitive.

**Lead.**—The test for lead is best carried out in the presence of potassium cyanide, when none of the other heavy metals react with dithizon. For lead the colour change is from green to red. The smallest amount recognisable in a drop of 0.05 c.c. is 0.1–0.2 $\gamma$  of lead, according to the other ions present. The test can be carried out in the presence of silver, copper, nickel, zinc, cadmium or antimony in more than 10,000 times the concentration. Ammonium chloride should be added when zinc or cadmium is present.

**Silver** gives a characteristic violet precipitate. The test is best carried out in dilute sodium hydroxide solution; in 4 per cent. sodium hydroxide the amount detectable is 0.3 $\gamma$  of silver. A little Rochelle salt should be added to prevent hydroxide precipitation.

**Zinc** colours the reagent purple red; the test may be carried out in 10 per cent. acetic acid solution, when 0.9 $\gamma$  of zinc may be detected, or in the presence of 10 per cent. acetic acid and 5 per cent. ammonium acetate, when 0.05 $\gamma$  of zinc can be detected. The test is not successful in the presence of silver, mercury, cobalt or much copper, though nickel and small amounts of copper may be present.

**Cobalt.**—The test for cobalt is carried out in 2 per cent. ammonia solution, when 0.04 $\gamma$  of cobalt is detectable, but in the presence of zinc a solution of dithizon in sodium hydroxide is used. With zinc alone there is a blue-violet colour, turning to grey in a few minutes, but in the presence of cobalt the colour retains its intensity for some hours, so that less than 0.1 per cent. of cobalt in zinc can be detected in this way.

J. W. B.

**Tests for Zirconium. F. Pavelka. (Mikrochem., 1930, 8, 345–351.)—**

**Carminic Acid Test.**—The yellow acid solution of carminic acid gives a permanganate-coloured compound with zirconium ions. The test solution should be acid (4–5 drops of 2 N hydrochloric acid per c.c.), and a dilute carminic acid solution is added; the colour change is visible after a few minutes on the water-bath. The smallest amount recognisable is 10 $\gamma$  of zirconium in a dilution of 1:100,000. The colour for 20 $\gamma$  is approximately that of N/1500 potassium permanganate. In the presence of thorium, cerium and aluminium the solution should contain 20 per cent. by volume of concentrated hydrochloric acid. Titanium interferes with the test, and also sulphates, phosphates and fluorides.

**Gallocyanin (dimethyl-amino-hydroxy-oxazon-carboxylic acid) Test.**—Zirconium changes to blue the red coloured solution of gallocyanin in acid. The test is carried out as with carminic acid, and 2 $\gamma$  of zirconium can be detected in 1:500,000 dilution. In the presence of aluminium, thorium, beryllium or cerium 10 per cent. of hydrochloric acid should be present. Titanium interferes. When the gallocyanin complex is precipitated with sodium arsenate solution (4 per cent.) the precipitate formed is distinctly blue, and can be concentrated by filtering through an Emich filter stick with an asbestos filtering surface, and, on comparing the colour with a blank, 2–3 $\gamma$  of zirconium can be recognised by giving a distinct blue colour. The gallocyanin compound can also be precipitated with *m*-nitro-phenyl-arsenic acid, when 5–10 $\gamma$  of zirconium can be detected in the presence of

500 times the amount of beryllium and thorium, but titanium interferes. By filtration, as before, as little as 1% can be detected by comparing the colour of the asbestos filtering mass with a blank.

J. W. B.

## Physical Methods, Apparatus, etc.

**Electric Heater for Pregl's Micro Combustion. B. Flaschenträger.** (*Mikrochem.*, 1931, 9, 15-19.)—An improved and simplified electric heater for the micro-combustion is made up of three parts, the large heater for maintaining the oxidising portion of the tube at a red heat (which uses about 800 watts), a small movable heater for burning the substance (using about 300 watts), and a low-temperature heater for heating the portion of the tube containing the lead peroxide (using about 100 watts). The low-temperature heater may be removed for the combustion of nitrogen, sulphur or halogens. Platinum foil, which is cheaper and lasts longer, is used in the heaters, instead of platinum spirals. The apparatus is obtainable from Heraeus. The advantage of electric heating over gas heating for the micro combustion is that constancy in temperature and pressure of the combustion space, which are important on the small scale, are more easily obtainable.

J. W. B.

**Nature of the Dust in the Air of Cotton Card Rooms. The British Cotton Industry Research Association.** (*J. Textile Inst.*, 1931, 21, 595-604T.)—The paper is an abridgment of the Report made in April, 1928, by the British Cotton Industry Research Association to the Federation of Master Cotton Spinners' Associations, and is based mainly on investigations by T. B. Bright, E. Rhodes, and F. Summers. The dust produced during the carding of Indian, American and Egyptian cottons in 17 card rooms, covering as wide a range of practical conditions as possible, was investigated and was found to be mainly made up of very minute particles with an admixture of very large particles. About 1 per cent. of the total dust might consist of fungus spores, especially those of the *Aspergillus niger* group. The living organisms were collected by aspirating 50 litres of air through a plug of anhydrous sodium sulphate contained in a glass tube of internal diameter  $\frac{3}{8}$  in. and length 8 in., and which was placed above a slight constriction about 2 in. from one end, with a plug of sterilised bacteriological cotton wool above. Two 10-litre aspirators were used alternately. The sodium sulphate was dissolved in 1 litre of normal saline, and 1 c.c. of solution transferred to agar which, after cooling, was incubated. Cultures were also made at dilutions of 1/10 and 1/100. Beer-wort agar at 25° C. was found the most suitable medium. The ratio of organisms of all kinds to total dust did not vary greatly from test to test, but the spores of *Aspergillus niger* rarely formed less than 50 per cent. of the total spores, and at times exceeded 90 per cent. The dust was collected for chemical analysis by passing the air through a scrubber consisting of a pair of 2-litre aspirator bottles with a large filter flask as a trap between the second and the vacuum pump. The bubble stream was very fine, this being ensured by causing the air to pass through the pores of a Gooch crucible fitted into the neck

of the second aspirator. After collection, the water from the apparatus was gently boiled; 1 per cent. of formalin added; and the dust collected on a tared filter paper and dried and weighed (the part soluble in water was neglected). After ashing and weighing, the silica was fixed with hydrochloric acid and determined. About 90 per cent. of the dust consisted of organic matter (cotton hair fragments, seed coat particles and fungus spores) and approximately 60 per cent. of the inorganic matter was silica. The kind of cotton or the method of mixing does not very much affect the character of the dust, but the quantity of particles and proportions of the different kinds of particles vary; *e.g.* Egyptian Uppers gave rise to more black mildew spores than the Texas sample. D. G. H.

## Reviews.

A TEXT-BOOK OF ORGANIC CHEMISTRY. By A. F. HOLLEMAN, Ph.D., LL.D., D.Sc., F.R.S.E. Seventh English Edition. Pp. xx+594. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Limited. 1930. Price 17s. 6d. net.

To those who welcomed the first English edition of Hollemann in 1902 it will be no surprise to find that seven English editions have been called for in less than thirty years. It is generally taught that Wöhler's synthesis of urea in 1828 broke down the distinction between inorganic and organic chemistry; one thing it did not do, break down the division between the ways in which the two branches of the science were taught. The ideas and methods of physical chemistry were soon utilised by the inorganic man; the organic chemist may have realised their importance, but hardly introduced them into his teaching. In these days of "indeterminancy" it is unwise to insist too strongly on cause and effect; it may be remarked, however, that the realisation of the industrial synthesis of the simpler "organic" compounds, such as methyl alcohol, urea and acetic acid, seems to be due to a combination of physical chemistry and engineering. The first edition of Hollemann departed from the traditional method of teaching organic chemistry, and in the present edition it will be noted how physical ideas are continually introduced. Thus, the reproduction of a few fractional-distillation curves on p. 22 warns the reader immediately as to the limits set to separation by distillation, and renders subsequent reference to "hydrates" of some of the lower alcohols unnecessary.

The book is divided into three main portions: An Introduction (pp. 1-32), First Part, The Aliphatic Compounds (pp. 33-348), and Second Part, Cyclic Compounds (pp. 349-560). The short introduction deals with the history of organic chemistry and successively with qualitative and quantitative analysis

and the determination of molecular weight. Half a page (p. 17) is devoted to "the element carbon." This is a liberal allowance, as organic text-books go, but one sometimes feels that it would not be illogical if a Text-book of Organic Chemistry began with a short account of the properties of the element carbon, both physical and chemical. Even the diamond and graphite lattices and the water-gas equation might be included, let alone a list of the elements with which carbon can enter into direct combination. The remainder of the introduction is concerned with laboratory methods, *e.g.* distillation, separation of solids and liquids, the polarimeter, etc.

On turning to the First Part, we meet first the alkanes,  $C_nH_{2n+2}$ , as Professor Hollemann systematically calls the paraffins. These are dismissed somewhat briefly, but serve as a convenient peg on which to hang an account of isomerism and structure. In dealing with the alkanols (alcohols,  $C_nH_{2n+1}OH$ ) it is possible to introduce the subject of optical activity (pp. 57-61), and in much the same way strength of bases (p. 74), velocity of formation of tetra-alkylammonium iodides (p. 77), melting-points of homologues (pp. 94 and 178), viscosity and fluidity (p. 97), surface-tension of soap solutions (p. 100), electrolytic dissociation and strength of acids (pp. 102, 191), ester equilibrium (pp. 107-111), molecular refraction (p. 154), formation of lactones (p. 207), thermal analysis (p. 372), etc., are dealt with as occasion offers.

For the rest, the First Part is a clear and concise account of the chemistry of the fatty series, stereochemistry and tautomerism being dealt with as occasion arises. Special attention may be drawn to the sections (pp. 231-281) dealing with the carbohydrates. They occupy about one-eleventh of the whole book, and for this, justification is to be found in the interest taken in the subject and the way in which the author has treated it. The proteins receive about 12 pages and the uric acid group about 8 pages, so that those parts of chemistry dealing with life receive fair treatment. Attention may be drawn to the special articles devoted to "Cyanogen Derivatives" and "Derivatives of Carbonic Acid"; they are to be recommended.

The aromatic series is treated on very sound lines, though more space for the Vegetable Dyestuffs and Tannins would have been welcome. The Hydrocyclic (hydro-aromatic) Compounds are allotted 20 pages, and included in this small space are accounts of the *cis-trans* isomerism associated with this series, and of the more important terpenes and camphor.

The space given to Heterocyclic Compounds is only 38 pages, and this has to suffice for pyridine, furan, pyrrole, thiophene, pyrazole, quinoline, *iso*-quinoline, indol (including indigo) and their derivatives, as well as for the alkaloids. It is not enough, and when a new edition is called for, it is to be hoped that the heterocyclic compounds will get rather more space.

The book is well-written and full of information, the printing and general appearance are good, and the index is adequate. Misprints are very rare. As a general text-book of organic chemistry the work may be very heartily recommended.

J. T. HEWITT.

**THE MANUFACTURE OF ARTIFICIAL SILK (RAYON).** Second edition. By E. WHEELER, M.B.E., A.C.G.I., F.I.C. (Monographs on Applied Chemistry, Vol. I.) Pp. xi+177. London: Chapman & Hall. Price 12s. 6d.

This book is one of a series of monographs on applied chemistry, and in attempting to assess its value it is, perhaps, worth while to examine the aim behind the series. Dr. Howard Tripp, whose task it is to act as editor for the series, clearly indicates in the preface his ideal, for he says that in looking on "scientific discoveries and their application to human welfare" it becomes necessary to attempt an evaluation of their real worth. He suggests, and with justice, that our scientific journals are "being swamped by a mass of second- and third-rate material that is thought to be worth publishing, but which posterity will decree would have been better left in manuscript form," and he has designed this series of monographs with a view to suppressing the chaff and presenting the germ in an accurate, assimilable and attractive form. As a sample of the series, Mr. Wheeler's book has, beyond doubt, fulfilled this aspiration, and justification of this is afforded by that delight of all authors—a second edition.

The present volume has not departed to any appreciable extent from the first volume except, of course, that it has been brought up to date in a very thorough manner. As is indicated in the sub-title, special reference is made to viscose silk, 50 pages being devoted entirely to the manufacturing details of the process. At the same time, the three other processes which are used commercially to-day are not by any means neglected, and, in all of these, the steps in the processes of manufacture are very concisely and clearly described. References are also made to practically all the attempts which have been made to produce artificial silks from other materials, as well as to the latest work of Lilienfeld on esters of cellulose.

Two chapters are devoted to the properties of artificial silk, one of which deals with the intricate business of dyeing. In dealing with this aspect of artificial silk, the author retains his aloofness from theoretical discussion, which characterises the whole book, although just once, on p. 129, he alludes to the theoretical work of Ellis on the effect of different acid groups in the dyestuff on the affinity of the dye for cellulose acetate.

From the analyst's point of view, the appendix will prove attractive, since the author describes suitable methods for controlling the manufacture of viscose, for testing the physical properties of the silk, and for the identification of the different varieties.

The book must be looked on as an excellent survey of the manufacture of rayon, with a sufficiency of recipe to enable a chemist to perceive the general lines on which the industry works. The English is fluent yet concise, and the printing, including a number of interesting photomicrographs and pictures of plant, well done. It is not, however, a book to stimulate the imagination. R. H. MARRIOTT.

**CHEMICAL ANALYSIS OF SPECIAL STEELS.** Fourth Edition. By C. H. JOHNSON. Pp. 721 and index. New York: Wiley. London: Chapman & Hall. Price 37s. 6d.

This book—"Johnson"—is well known in all laboratories that deal with



metallurgical materials, and the previous edition has been expanded to include 18 appendices, amounting to 176 additional pages with 28 new illustrations. The book is presented on good paper and in clear readable type, while the appendices, which include new data and modern methods of analyses, are printed in smaller type. This is meant to be a comprehensive text-book, but it is doubtful whether this method of presentation of a new edition will be a popular one, as the information given is very scattered; yet a complete resetting of the third edition "would probably never have been done."

The author is clearly an enthusiast and realises *ab initio* that modern conditions demand a specialised knowledge in the realm of alloy steels, and he provides for most of the materials that are likely to find their way into the modern steel works' laboratory. Much useful practical information is given, while it is claimed that "all methods are developed in extreme detail purposely for the benefit of the novice." This is true, as the book contains methods that are repeated "in extreme detail" with monotonous repetition throughout, while, since the author has attempted to bring the methods of analyses up to date, it seems unfortunate that the old stereotyped methods are still quoted in such minute detail, and that important developments even in steel analysis, that have taken place during the past ten years have been completely ignored.

It has been proved by Clarke (ANALYST, 1927, 52, 466) that in the determination of vanadium in a tungsten steel the tungstic acid retains notable amounts of the element, but in the method described on p. 7 the author does not recover this occluded vanadium, and the excellent method of a direct precipitation of the metal as the ferrocyanide (Evans and Clarke, ANALYST, 1928, 53, 475), which obviates the necessity of separating tungsten, has been overlooked. In the determination of phosphorus as ammonium phosphomolybdate (pp. 310, 522, etc.) the interference of chromium, arsenic, silicon, etc., has not been considered, and in the determination of sulphur in alloy steels the author is in the happy position of being able to dissolve the materials in *concentrated* nitric acid (pp. 335-7), and does not consider high chromium steels of the well-known stainless variety, which are not decomposed by either nitric acid or *aqua regia*. In this country the direct "bismuthate" method for manganese in steels containing less than 2 per cent. of chromium is deservedly popular, in preference to the use of peroxide of lead, as on p. 601.

It is stated on p. 56 that "there is much demand for the determination of chromium in steels with less than 0.10 per cent. Cr," and that "all plain carbon steels without exception contain from 0.01-0.1 per cent. of nickel and chromium" (p. 604), yet the author does not appear to be aware of the well-known method of Evans (ANALYST, 1921, 46, 38), which is used in modern laboratories for the accurate determination of traces of chromium in steels. The book, in fact, is sadly lacking in suitable colorimetric methods for such elements as nickel, copper and cobalt in steel,\* bismuth in lead alloys, etc., where large weights of sample are employed by the old methods of analysis (pp. 209, 605, 688). There are better

\* Cf. ANALYST, 1930, 55, 318.

methods for the determination of lead in copper alloys than the unsatisfactory separation and weighing as the sulphate (p. 662), while the separation of copper in these alloys as sulphide (p. 663) is an old procedure that has been generally replaced by the cleaner and more accurate electrolytic separation, as described by Etheridge (ANALYST, 1924, 49, 371). Methyl orange is not sufficiently sensitive as an indicator for very dilute solutions in acidimetry titrations, as described, for example, on p. 653.

A good feature of the book is that the results of test analyses, together with representative analyses of many materials, are given, and many useful references to original papers—mostly American—are quoted. Chemists will welcome the chapter on such matters as the testing of lubricating oils (Chap. XIX) and on metallurgical practice, such as the microscopic examination of steel (Appendix XVI), heat treatment and "spheroidising" (Appendix XVIII).

The English is poor in parts, and the constant reiteration of the words "proven" and "gotten" will irritate English readers. The author has a curious nomenclature of his own, coining expressions, for example, such as "adding a slight excess of *ammonium*" (pp. 48, 52, 111, etc.), "*ammonia* salts" (p. 411), "fill the tube with the *H*" (p. 184), "*H Fl*" (p. 193), "8 per cent. *Ti ferro*" (p. 64). These are not to be recommended, while instructions to "dissolve 200 mgs. of steel" (p. 16, etc.), "170 mgs. of potassium dichromate" (p. 18), etc., will appear rather confusing to the novice. Several errors in printing may be noted, e.g. "*anealing*" (p. 423),  $KClO_3$  (p. 592, etc.),  $HNO$  for  $HNO_3$  (p. 596),  $Cr_2O_3$  for  $Cr_2O_3$  (p. 623), a saturated solution of  $BaSO_4$  for  $BaCl_2$  (p. 177); the number of a page is omitted in a reference at bottom of p. 377.

Many of the methods described are excellent, however, and have been tested for accuracy, and the book is, in effect, an encyclopaedia of knowledge to the metallurgical chemist.

B. JONES.

I SOFFIONI E I LAGONI DELLA TOSCANA E LA INDUSTRIA BORACIFERA. By RAFFAELLO NASINI. Pp. xi+658. Rome. 1930.

The publication of this sumptuous volume, which is the work of Senator Nasini and various collaborators, has been rendered possible by the generosity of Prince Piero Ginori Conti, to whom the industrial utilisation of the heat furnished by the soffioni of Tuscany is so largely due. The introductory chapter consists of a lecture delivered in 1923 by Nasini, whose acquaintance with Lardarello began as long ago as 1877. A very complete account is next given of early references to the soffioni and lagoons (corrupted from the Latin *lacunae*). These were known to contain sulphur, alum, salt and vitriol, and the mineral wealth of the district in which they occurred was recognised, long prior to the discovery of their content of boric acid, known as Homberg sedative salt, in 1777 by Hoefer, who then held the position of druggist to the Grand Duke of Tuscany. Hoefer detected the acid by the green colour it imparted to a spirit flame.

During the early years of the nineteenth century considerable progress was made in the methods of extraction, the development of a flourishing industry being

due mainly to the exertions of Count Francesco De Lardarel (1789–1858). Notable improvements were effected by the introduction of so-called Adrian boilers for concentrating the clarified lagoon waters and by that of artificial soffioni and artesian borings, suggested in 1840 by Professor Gazzeri. In 1912, the whole of the factories came into the possession of Count Florestano De Lardarel and Prince Ginori Conti, who founded the powerful "Società Boracifera di Lardarello." Since that time great strides have been made in various directions, notably by the installation of an enormous plant for the liquefaction of the carbon dioxide issuing in vast quantities with the steam, the recovery of argon and helium, and the improved utilisation of the steam, of which more than 60,000 kilos per hour is available.

The bulk of the work, occupying 500 pages, consists mostly of an account of the large amount of scientific work carried out during the past twenty-five years by Nasini and his co-workers on boric acid and other boron compounds, and on subjects suggested by a study of the soffioni and lagoons.

The printing and form of the volume are irreproachable, the numerous illustrations are well reproduced, and the matter included is of great practical and historical interest. The book is not on sale. T. H. POPE.

ANLEITUNG ZUR ORGANISCHEN QUALITATIVEN ANALYSE. By H. STAUDINGER. 2nd Edition. Pp. xv+144. Berlin: J. Springer. Price M. 6.60.

This practical little text-book, which deals with the qualitative analysis of organic compounds and mixtures in a systematic manner having some analogy with the systems of inorganic analysis, has deservedly reached its second edition within the space of six years.

The introductory section, which forms the theoretical basis of the work, has been considerably enlarged, although still following the original classification into main groups in accordance with volatility, melting point and solubility, and the separation of these groups into subsidiary groups. The analytical scheme for the separation of mixtures is also given in the form of tables, which are the work of Dr. W. Frost.

In the special sections, on the separation of organic compounds, as classified into chemical groups, the various sub-divisions are also based on physical properties, and there are copious tables of the recorded data for the identification of individual substances (*e.g.* melting points of mixtures).

The actual methods of determining physical constants and molecular weights are not described, as the book is intended to supplement, not to replace, the ordinary text-books on practical organic chemistry.

As a laboratory guide it can be warmly recommended, not only to students, but also to analysts whose work involves the examination of mixtures of unknown organic substances. EDITOR.

# THE ANALYST

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## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

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THE Annual General Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, March 4th, when the President, Dr. J. T. Dunn, delivered his Annual Address.

The following were elected as Officers and Council for the year 1931:

*President.*—Dr. J. T. Dunn.

*Past Presidents, serving on the Council.*—E. Richards Bolton, A. Chaston Chapman, Bernard Dyer, Edward Hinks, P. A. Ellis Richards, G. Rudd Thompson, J. Augustus Voelcker.

*Vice-Presidents.*—A. More, W. Partridge, G. Roche Lynch.

*Hon. Treasurer.*—E. B. Hughes.

*Hon. Secretary.*—F. W. F. Arnaud.

*Members of Council.*—A. L. Bacharach, F. H. Carr, C. H. Cribb, H. E. Cox, E. M. Hawkins, A. E. Johnson, D. W. Kent-Jones, H. M. Mason, W. G. Messenger, G. W. Monier-Williams, G. Stubbs, A. R. Tankard.

An Ordinary Meeting of the Society then followed, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—K. N. Bagchi, B.Sc., M.B., D.T.M., William Nelson Bradshaw, B.Sc., Adrian Joseph Clifford Lickorish, F.I.C., Ernest Grenville Purser, B.Sc., A.I.C., and William Waddell Robson.

Certificates were read for the second time in favour of:—Cecil Chilvers, B.Sc., F.I.C., Jack Hubert Hamence, M.Sc., A.I.C., Cecil John House, B.Sc., A.R.C.Sc., F.I.C., and Henry George Rees, B.Sc., A.R.C.Sc., A.I.C.

The following were elected Members of the Society:—Kenneth Bullock, M.Sc., Ph.D., and Frederick Cecil Hymas, B.Sc., A.I.C.

The following papers were read:—"The Investigation of Japanese Beeswax," by H. Ikuta; and "The Denigès-Oliver Test for Morphine," by J. Bamford.

# Annual Report of Council

March, 1931.

THE Roll of the Society stands at 661, an increase of 41 over the membership of last year.

With great regret the Council has had to report the death of:

Edward William Voelcker, who served the Society as Treasurer for many years, and as President during 1910-1911.

The Council also deplores the loss of two honorary members and three ordinary members:—

H. W. Wiley ("Obituary," ANALYST, 1930, 55, 728).

Ludwig Moser.

Arthur Angell ("Obituary," ANALYST, 1930, 55, 308).

Henry Leffmann.

A. MacLean Wright.

During the year, seven meetings of the Society were held, and the following papers were communicated :

- "The Determination of Minute Amounts of Iodine in Soils and Waters." By R. L. Andrew, F.I.C.
- "Preliminary Studies in the Bacteriology of Wheat and Flour." By D. W. Kent-Jones, Ph.D., B.Sc., F.I.C., and A. J. Amos, B.Sc., A.I.C.
- "The Separation of Metals by 'Internal Electrolysis'." By H. J. S. Sand, D.Sc., Ph.D., F.I.C.
- "The Rapid Determination of Bismuth and Copper in Lead Bullion by Internal Electrolysis." By Ella M. Collin, B.Sc., A.I.C.
- "Notes on the Thiocyanate Method of Determining Iron. Influence of Different Classes of Phosphates." By G. Winthrop Leeper, M.Sc.
- (i) "The Spectroscopic Investigation of Jams and the like." (ii) "A Simple Polarimetric Test for Sugars in Jams." (iii) "A New Kjeldahl Distillation Apparatus." By S. Judd Lewis, D.Sc., F.I.C.
- "The Determination of Cadmium and Copper in Spelter and Zinc Ores by Internal Electrolysis." By Ella M. Collin, B.Sc., A.I.C.
- "The Routine Detection of Nitrates in Milk." By A. F. Lerrigo, B.Sc., F.I.C.
- "A Method for the Determination of Titanium as Phosphate." By J. C. Ghosh, D.Sc.
- "The Diastatic Activity of Honey." By L. H. Lampitt, D.Sc., F.I.C., E. B. Hughes, M.Sc., F.I.C., and H. S. Rooke, M.Sc., A.I.C.
- \*"A New Method for the Separation of Titanium from Zirconium and Hafnium." By A. R. Powell and W. R. Schoeller, Ph.D.
- "The Composition and Polymerisation of Chinese Wood (Tung) Oil." By E. R. Bolton, F.I.C., and K. A. Williams, B.Sc., A.I.C.
- "The Examination of Milk for Tubercle Bacilli." By D. R. Wood, F.I.C.
- "Scientific Evidence relating to Firearms, with Special Reference to a Recent Murder Trial." By G. W. Baker, F.I.C.
- \*"The Composition of Rye Oil." By J. W. Croxford, A.I.C.

\* Work done under the Society's Analytical Investigation Scheme.

- "The Determination of Unsaponified Oil in Soap or Fatty Acids." By G. E. Lester Smith, M.Sc., A.I.C.
- "The Analysis and Composition of Vegetable Parchment used for Packing Dairy Products." By Paul Arup, M.Sc., F.I.C.
- "The Determination of Milk Proteins." By George M. Moir, M.Sc., Ph.D., A.I.C., Pedler Research Scholar of the Institute of Chemistry.
- "The Lead Reduction Method for the Volumetric Determination of Tin, and the Interference by Copper and Antimony with it." By S. G. Clarke, B.Sc., Ph.D., A.I.C.
- "A Storage and Delivery Apparatus for Antimony Chloride and other Corrosive Reagents." By G. Middleton, B.Sc., A.I.C.
- "Tests for Impurities in Ether." Parts II and III. By G. Middleton, B.Sc., A.I.C., and F. C. Hymas, B.Sc., A.I.C.
- "The Determination of Small Quantities of Calcium in Magnesium Salts." By Norman Evers, B.Sc., F.I.C.
- "A New Method for the Detection of Nitro-Group in Organic Compounds." By P. K. Bose, D.Sc.

The following papers were read at meetings of the North of England Section:

- "Drinking Waters for Cattle." By T. MacLachlan, F.I.C.
- "The Freezing Point of Milk as a means of Detecting added Water." By G. D. Elsdon, B.Sc., F.I.C., and J. R. Stubbs, M.Sc., F.I.C.
- "Gas Fatalities due to the Slow Oxidation of Vegetable Refuse." By A. R. Tankard, F.I.C., and D. J. T. Bagnall, A.I.C.
- "The Examination and Commercial Analysis of Cotton Cloths." By R. H. Kay, A.T.I.
- "The Reichert-Polenske-Kirschner Values of Rancid Butters and Margarines." By G. D. Elsdon, B.Sc., F.I.C., R. J. Taylor and P. Smith.
- "The Detection of Benzoic Acid in Food." By A. N. Leather, B.Sc., F.I.C.

The pages of the ANALYST also contained a considerable number of other original papers and notes.

The Editor of the ANALYST, Dr. C. A. Mitchell, reports an increased sale of the journal, the size of which equalled the pages in the volume for the previous year, which was the largest volume published up to that date.

Reference to the Treasurer's statement, which is circulated to members separately, shows that expenses, as in the past, have been successfully met.

Reports were received from three of the Sub-Committees of the Standing Committee on Uniformity of Analytical Methods, namely :

Essential Oils Sub-Committee (two Reports).

Milk Products Sub-Committee.

Metallic Impurities in Food Colours Sub-Committee.

These have been published in the pages of the ANALYST. They have necessitated a very large amount of work by members of Sub-Committees, and an expression of appreciation of the work was forwarded, on behalf of the Council and of the Society, to the Standing Committee.

The Council express their appreciation of the facilities afforded to members of the Society for the use of the library of the Chemical Society during the year.

The Council received a communication from the Association of British Chemical Manufacturers with regard to the formation of a British Chemical Standardising Body. The Society was represented at a Conference at which this question was discussed, and a small Committee was appointed by the Conference to report to them.

A representative International Committee met at Geneva to discuss International Methods of Oil Analysis, Mr. E. R. Bolton being appointed representative of the Society. The Committee decided that a Conference should be held in different countries annually, and that each country should be represented by one individual only.

A Dinner held by the Society on the 4th March, at the Savoy Hotel, was largely attended and very successful. Among the many important guests were :—The Rt. Hon. Arthur Greenwood, M.P., Minister of Health ; The Rt. Hon. Lord Tomlin, Lord of Appeal ; The Rt. Hon. Lord Riddell ; and the Presidents of the Chemical Society, the Institute of Chemistry, the Society of Chemical Industry, the Institute of Brewing, the Institution of Chemical Engineers, and the Institution of Petroleum Technologists.

A letter was received from the Ministry of Health asking for observations with respect to the standardisation of glassware for testing milk and milk products. The Council supported the specifications contained in the publication of the National Physical Laboratory issued in April, 1927, but at the same time expressed the view that it did not approve of centrifugal methods for the determination of fat in milk, except as approximate methods.

A Cheese Bill was presented to Parliament. The composition of cheese had consequently been considered by the Council, who made the following proposals :

- (1) That a statutory standard for fat in cheese was desirable.
- (2) That the legal minimum of fat in cheese should be 45 per cent. of milk fat in the dry substance.
- (3) That all cheese made from skimmed milk should be labelled "Skim Milk Cheese," with a further declaration of "three-quarter fat," "half fat," or "one-quarter fat," according to the proportion of milk fat contained in the milk from which the "Skim Milk Cheese" was prepared. Minimum limits for the fat occurring in the dry substance of "three-quarter fat," etc., "Skim Milk Cheese" should be laid down.

The Conference of Members of the Food Manufacturers' Federation and Public Analysts has issued its report on the standards for jam, and these standards have been circulated in the form of a final memorandum. This has also been published in the ANALYST.

J. T. DUNN, *President*.

F. W. F. ARNAUD, *Honorary Secretary*.

## Annual Address of the President.

(DR. J. T. DUNN, F.I.C.)

*Delivered at the Annual General Meeting, held on March 4, 1931.*

LADIES AND GENTLEMEN,

It has long been customary for the President to begin his address by an account of the position of the Society in membership and activities, quoting statistics and enumerating papers read during the year. All this is contained in the report of Council, and it seems unnecessary to repeat it in detail; but it is gratifying to find that we are steadily gaining numerical strength in membership. Both this circumstance, and the fact that the sales of *THE ANALYST* are increasing, are testimony to the excellence of our journal, and a tribute to the solid and careful work of the Publication Committee, and especially to the ability and untiring industry of our Editor, Dr. Mitchell, to whom the Society owes so much.

A Society like ours, which has passed its jubilee, must expect every year to have to record the passing of some of its older members; and this year is no exception to the rule. But of those who have come within "Time's bending sickle's compass," whose names we see in the Council's report, and among whom are some of considerable eminence, particularly in connexion with the analysis of foods, there is one in especial whom I must mention, because of his long and intimate association with our Society. Edward William Voelcker was elected a member in April, 1889, forty-two years ago; and during the whole of that long period, as member, as Member of Council, as President, and, above all, as Treasurer, he has given of his best to the Society. His financial ability, and the wisdom and moderation of his counsel—ability and wisdom never blatantly displayed, but perhaps even a little hidden from immediate recognition by the modesty of his demeanour, and the geniality and kindness of his nature—have undoubtedly largely helped towards the growth of the Society and the attainment of its present prosperous position. We miss not only his ready help and advice, but his personal charm, which all of us must have felt. He is become for us a fragrant memory, and the contemplation of his life and work cannot but be a stimulus and an inspiration.

The papers read during the Session are enumerated in the Council's report. I should like here only to draw attention to the wide range of the subjects treated in them, and to point out that almost half of them have no connection with Food and Drugs or the official work of the Public Analyst. The subjects cover practically the whole range of analytical chemistry, and testify to the wisdom of the policy which, a few years ago, added "Other Analytical Chemists" to the membership of the Society of Public Analysts.



**THE NORTH OF ENGLAND SECTION.**—This Section, I am glad to think, continues a vigorous existence with an increasing membership, and is becoming an affluent towards the membership of the parent Society. It held last July a week-end summer meeting at Scarborough, which was well attended and very successful. Glancing through old volumes of the ANALYST, I see that the Society was in the habit of holding a country meeting once a year, and it is worth while, I think, asking whether the practice might not usefully be revived. The opportunities for social intercourse which such a meeting gives are very valuable. Another feature of the Section's session was a visit to one of the factories of the Co-operative Wholesale Society, who entertained the members to lunch and tea; which indicates that the C.W.S. was not ashamed to submit its processes to the gaze of the Public Analysts, nor its products to the judgment of their palates.

**CO-OPERATION WITH MANUFACTURERS.**—The Council's report for last year mentioned that, at the invitation of the Food Manufacturers' Federation, conferences between their representatives and the Public Analysts' Committee were taking place, on the question of standards for jam, and you now know that agreement has been arrived at, and that a memorandum has been circulated to the members of the Federation, setting forth the standards to be adhered to, and the mode of labelling the jam. All of the members of the Federation have signed the declaration agreeing to abide by these standards; and I take it that our position as Public Analysts will be to look upon any sample of jam, not labelled "Lower Fruit Standard," as purporting to be "Full Fruit Standard," and to report against it if it should fall short of the requirements of that standard.

I regard this arrangement as of very great importance and with a considerable degree of satisfaction. In the early days of the Food and Drugs Act, and indeed for many years after its institution, the Public Analyst was looked on as more or less the natural enemy of the manufacturer—the earlier volumes of the ANALYST contain frequent references to this attitude—and any sort of co-operation between them would have been thought almost an impossibility. Gradually, as the various branches of the manufacture of food products have become more scientific—as manufacturers in increasing numbers have themselves called in the aid of the chemist to control and extend their work, and have more and more realised that success lies in the direction of raising the standard of excellence of their wares rather than in actually (though not professedly) lowering it, that feeling has died out; and of late years, and especially since the chief producers of different food products have banded themselves into associations, and have seen that it is to their advantage not only to keep up the standard of their own products, but to endeavour to prevent others of lower morality from spoiling their markets by the presentation of inferior or adulterated articles at lower prices, we find the manufacturers helping the Public Analysts, and in turn asking for their help. Three years ago we had the chemists of the Food Manufacturers' Federation placing unreservedly before us the results of their investigations into methods for the determination of the preservatives used in foodstuffs; and now we have the

Federation asking for our co-operation in fixing reasonable standards of quality for jam.

And this example seems likely to be followed. During last year the National Farmers' Union and the Cheshire County Council endeavoured to promote a bill in Parliament with the view of checking the sale in this country of inferior cheeses as Cheshire cheese; and they approached your Council to know whether or not it would give them backing. The Public Analysts' Committee considered the Bill, and, whilst giving it general approval, suggested alterations and additions for its improvement, and their proposals were approved by Council. No definite result has yet been arrived at, but the fact that the help of the Society was sought by the producers is very significant.

Quite lately the Council has been approached by another branch of the Food Manufacturers' Federation to see whether agreement can be arrived at on the question of rice flour in suet. The Public Analysts' Committee is now considering this, and I can, of course, say nothing on the question itself, but again chronicle the fact of the producers' endeavour to work in harmony and co-operation with the Public Analysts.

If these conferences continue and spread, we may be within measurable distance of having, not, indeed, legal standards, but at least agreed standards of composition of many foodstuffs, which will acquire in the courts the force and authority of legal standards; and we shall no longer have the anomaly of the same substance being held in one place to be genuine and in another adulterated.

And, as all this tends in the direction of informing the public as to what it is actually buying, I have hopes that it may go further yet, and that we may agree more upon definitions and descriptions; so that we shall not have tapioca sold as sago; shall know when we have a custard that it is not made from cornflour; and that a lemonade syrup shall not be said to be "made from the finest Messina lemons," if it has no nearer connection with them than the fact of its containing citric acid.

Our experience with the jam manufacturers showed that we can attain results in conference that no amount of written correspondence would reach. Each side was able to put before the other its point of view, of which previously the other side was quite oblivious, and which hence it was unable to appreciate. One, at least, of our proposals was met at first by direct and absolute refusal, and yet we were able to show that it was desirable, even in the jam manufacturers' own interest, and to carry their acceptance of it. And we learnt something of the difficulties of the manufacturers, when we found that things on which we had come to agreement with them raised strenuous opposition on the part of the retailers, to whom they sold their products. So, give and take were both necessary; and if the new situation is not quite ideal in the view of some of us, it is at least a working arrangement which marks a considerable advance on the state of things that preceded it, and gives us reasonable hope of further progress in the same direction.

The jam manufacturers and their research chemists found that in black-currant jam made from pulp it was very difficult, sometimes impossible, to get

down to the maximum permitted quantity of sulphur dioxide: 40 parts per million; and Professor Roberts, of Liverpool, and I, who had both had some experience, not only of samples taken under the Food and Drugs Acts, and containing more than the permitted quantity, but also of the honest but unsuccessful efforts of local jam manufacturers to comply with the regulations, had also come to the conclusion that the requirement was too stringent. Along with Mr. Macara and two of the jam manufacturers' chemists, therefore, we had an interview with Dr. Hamill at the Ministry of Health, and put before him facts and figures on the question. So far we have heard of no result of the interview, but I mention it as another illustration of friendly co-operation where formerly there was antagonism and distrust.

**CO-OPERATION IN FORENSIC WORK.**—Speaking of co-operation between manufacturers or their associations and analysts leads me to mention another aspect of work in which co-operation is extremely desirable, and, in my experience at least, frequently lacking. I refer to chemico-legal or toxicological cases, involving the examination for poisons of the organs of animals or of human beings. Here you have the veterinary surgeon or the medical practitioner and the analyst both involved, and it would seem eminently desirable that they should examine the organs together in the first place. But this is seldom done, and the organs are handed over to the analyst, frequently in a way that should not happen. I have two extreme cases in mind, both of them recent. In one we received a stomach for examination, the whole contents of which had been removed and the stomach washed. In the other, the whole of the intestines, with their contents, of a person supposed to have been poisoned, were sent to us, apparently without any previous examination. On opening them we found growths in such numbers and of such a size as seemed to us to be in themselves sufficient to account for death. My partner and myself were of course not competent to say definitely whether or not that was so; but had the medical man been present with us at the examination he could have given an authoritative opinion, and the search for poisons might not have been necessary. On the other hand, in another recent case, where the organs first came into the possession, not of a medical practitioner, but of a professor of pathology, he brought them to me and we opened and examined them together, so that each was furnished at the outset with the observations and inferences or suggestions of the other. It seems a great pity that this is not the usual practice.

**SALE OF MILK REGULATIONS.**—We are every now and then brought up against efforts of one kind or other to minimise the effective application, in the Courts, of the Sale of Milk Regulations. Last year's report of Council referred to a pamphlet of the Board of Agriculture, "Variations in the Composition of Milk," which was so obviously one-sided and unfair in its presentation and interpretation of the figures it contained, that your Council found it necessary to send the letter of protest that was published in the *ANALYST* of August, 1929. This year there was brought to the notice of Council, in the *Journal of the Ministry of Agriculture*, an account of a meeting in May of the "Council of Agriculture for England," at which, among other business, there was moved and adopted a report on the "Law

Relating to the Sale of Milk," which the mover said would be "brought to the notice of the Ministry of Health, with a view to seeing how far action could be taken in accordance with the spirit of the representation."

The report proposes to remove milk from the operation of the Food and Drugs Act, and to place it under a special Act. Under this Act the presumption of adulteration, which the vendor must rebut, when the milk falls below specified minimal contents of fat or of non-fatty solids, would disappear, and punishment under the criminal law would only be inflicted if wilful adulteration were actually proved by the prosecution (no specified penalties are suggested); but there would be an implied warranty that all milk sold should contain at least 3 per cent. of fat and 8·5 per cent. of non-fatty solids (though sellers would be at liberty to issue specific warranties guaranteeing either more or less than these quantities, presumably obtaining correspondingly higher or lower prices), and failure to maintain the standard guaranteed by the warranty would be a civil offence, punishable by the award of five shillings damages, if the case were proved in a County Court.

There is something to be said for the infliction of civil penalties only in certain Food and Drugs cases; and occasions do arise where a tradesman is branded with the stigma of criminal conviction under circumstances that do not warrant so severe and far-reaching a penalty. But the probable effect on the general milk-supply of the country of such an alteration in the law as is suggested in this report, where the maximum penalty, for what would always be a breach of contract and might frequently be deliberate fraud, would be "the award of five shillings damages," is very obvious. It is so obvious, I think, as to make the likelihood of the alteration ever coming into operation negligibly small; but I mention it because at the meeting in question the Minister of Agriculture was present, and in his speech said he "would like to emphasise how closely he and the whole Department attended to the recommendations and reports of the Council." Your Council is giving a watchful attention to the matter.

It is, perhaps, of interest to say that during last year I received a pamphlet from a large firm of food distributors on "Proposals for legislation amending the law as to the Sale of Milk." This made reference, apparently, to a former pamphlet which I had not seen, and went on to say: "We now put forward somewhat detailed proposals for milk legislation, which have been evolved. . . ." I read the pamphlet through, but took no further note of the proposals, in such a matter, of a private firm; but perusal of the report of the Council of Agriculture stirred up some dim remembrance in my brain, and I looked up the pamphlet again. I found, curious to say, that nearly the whole of the pamphlet is a verbatim transcription of the Council of Agriculture's report. I make no comment on the coincidence.

**STANDARD METHODS OF ANALYSIS.**—A year ago your President devoted a large part of his very interesting address to the question of standard methods of analysis. This is a question that from time to time has occupied the attention of the Society. I find that Mr. Rudd Thompson, in addressing you in 1925, referred

to standard methods that had then been adopted, here and abroad, and discussed the question generally; and Mr. Bolton, three years later, speaking of the same matter, said: "It will be clear to everybody that conditions have arisen requiring the institution of standard methods for certain purposes, and these standard methods must be provided," and criticised the Society for neglecting this work, and leaving it to be done, as in certain directions it was then being done, by the British Engineering Standards Association, an engineering body.

Last year your Society was asked by the Association of British Chemical Manufacturers to appoint delegates to a conference to consider the question of standards generally as applied to chemical products and to analytical methods, and Mr. Bolton and I duly attended on June 4th. The Conference agreed that the formation of a chemical standardising body should be attempted, and that it was desirable that such a body should form a part of a larger organisation dealing with all forms of standardisation rather than act independently; and a small committee was formed to explore the situation in conjunction with the B.E.S.A., and report to the Conference.

The Committee has just made its report, and recommends that there should be a general organisation to be called the "British Industrial Standards Association," and that it should consist of four divisions—Building, Chemical, Engineering, and Textile. Each division will be under the government of a representative council chosen from the bodies interested in its particular work, and, as far as that particular work is concerned, it will be autonomous, though it will be subject, in matters of general policy, to a general council, largely elected by the four divisions themselves. The constitution of the councils, and the relations between the divisional councils and the general council are still under discussion, and it would not be right for me to indicate the proposals which have so far been made in regard to them. I will only point out that, when the scheme is carried through, the chemical division, besides undertaking the standardising of substances or methods not yet dealt with, will take over for adoption or revision those parts of the work hitherto carried out by the B.E.S.A. which are chemical in their nature.

Whatever our views on standardised methods of analysis may be, we are forced to concede, I think, that under the present conditions of industry and commerce they are very frequently necessary, and that they are, in fact, insisted on by those who have the power to insist; and if we are to have them, it is all to the good that they should be elaborated by a general chemical body, on which this Society will always be able to make its voice heard; and which, as it prescribed the methods, will also be able to modify and improve them, as that may become necessary.

In parenthesis, I may perhaps express the pious hope that, when standardising is undertaken by a general chemical body, rather than by the particular industry concerned, more care will be exercised, especially where the methods of analysis, or of testing, involve the measurement of physical characters of one kind or another, to make sure, before insisting on a standardised instrument or form of apparatus,

that such a standardised instrument or form of apparatus is really necessary to obtain consistent results. Our friends, the Institution of Petroleum Technologists, for example, dealing with the question of the effect of cold on the viscosity of oils, prescribed for us a standard method of determining the "pour-point" of an oil, which involved a special apparatus. But in a second edition of their book they tell us that the "pour-point" is of no value; we must determine the "setting-point," which has no relation to, and gives results in no way comparable with those of the "pour-point" determination, but which requires another and different special apparatus. And they give us two alternative methods of inquiry into the carbonising qualities of an oil, embodying the names of Conradson and Ramsbottom, respectively. Some users specify Conradson; others, Ramsbottom. No doubt each gives results by which different oils can be usefully compared; but Conradson's results have no relation to Ramsbottom's, and the only feature common to both methods is that for each there is prescribed a special and expensive apparatus.

Now, to the firm or specialist who is doing a particular kind of work all day and every day, the purchase of a costly instrument to carry out the work is not very serious; but when the general practitioner is asked to carry out an operation which may not recur more than half-a-dozen times in a year, and finds that his results will not be accepted unless he has used Brown-Jones's automatic electropolar antiviscosimeter, or other prescribed instrument, which Messrs. Thermo and Muffle's latest gorgeous catalogue tells him will cost £45, and that there will be another £35 for the "accessories" that are necessary to make the instrument work, he is placed in a not very happy position, having to decide whether to refuse the work and perhaps lose a client who might give him other work besides, or to cut the loss, and risk getting repaid in other ways. It is quite possible for such a man to spend as much on apparatus as he makes in fees, and be left with nothing but the melancholy satisfaction of reflecting that he is serving his country, when he pays income tax on the income that he would have had, if it had not all gone in capital expenditure. That is perhaps an extreme instance; but I do seriously think that there is a strong tendency nowadays to rely too much on the instrument-maker, and too little on our own powers of manipulation, which tend, as a result, to become atrophied.

When you honoured me last year by offering me the presidency of the Society, I accepted it, though with great pleasure and pride at the thought that I was considered worthy of it, yet with some diffidence and trepidation; for, obviously, a President living so far away from London, and debarred from frequent personal consultation with the Secretary or the other members of Council, works under conditions of some difficulty; but I have had the loyal help of all the members of Council, whom I wish to thank for their uniform kindness, and among them perhaps I may without offence name especially my immediate predecessor, Mr. Hinks, for whose advice and assistance I am most deeply grateful. Mr. Arnaud, too, was most considerate, and spared himself no trouble in keeping me abreast of successive situations that arose, until he was laid off by that very severe illness, his recovery from which is matter for such sincere gratification to us all; and during

his enforced absence Dr. Mitchell took over his work and relieved me of the anxiety I might otherwise have felt at being bereft of a guide whom I felt I greatly needed. All this has made my year of office a time of most pleasurable memories. You have entrusted me with the office for a second year; I hope to carry out its duties acceptably; and should I fail, it will certainly not be for lack of either effort or inclination to fulfil them.

## The Determination of the Milk Proteins.

By GEORGE M. MOIR, M.Sc., Ph.D., A.I.C.,

*Pedler Research Scholar of the Institute of Chemistry, 1928-1930.*

*(Read at the Meeting, November 5, 1930.)*

### IV. A. THE COMBINED DETERMINATION OF ALBUMIN AND GLOBULIN.

OWING to the small proportion of globulin in milk most investigators have been content to precipitate the albumin and globulin together from the filtrate remaining after the removal of the casein. For very many years this separation has been effected by one of two processes, (a) by heat coagulation,<sup>1</sup> (b) by the use of Almen's tannic acid reagent. The fact that the first method gave distinctly low results was pointed out nearly thirty years ago by Simon,<sup>2</sup> and more recently by others<sup>3,4,5</sup>.

These investigators all recommended the use of Almen's reagent, but, unfortunately, tannic acid may precipitate other material besides the albumin and globulin. This possibility is evident from the reports of workers<sup>6,7,8</sup> whose experiments show that tannic acid may precipitate peptones and also certain amino-acids, traces of which may be present in fresh cows' milk. Directions<sup>14</sup> to report albumin and globulin as the difference between casein and total nitrogen, have probably led to even higher values being published. The tendency to high values has no doubt been accentuated also by the fact that casein cannot be completely removed by the use of acetic acid alone.

Other reagents which have been used for the removal of protein material are trichloroacetic, phosphotungstic and phosphomolybdic acids. Evidence is available<sup>6,9,10</sup> which favours the first of these.

Preliminary experiments were carried out in order to compare trichloroacetic and tannic acids as milk protein precipitants. For this purpose quantities of 20 ml. of the same milk were pipetted into beakers and treated in different ways. To A, 20 ml. of 10 per cent. trichloroacetic acid were added and the mixture filtered after standing about half-an-hour. To B, 20 ml. of 10 per cent. trichloroacetic acid were added, and the mixture maintained at a temperature of 60-65° C. for

half-an-hour before cooling and filtering. To C, 5 ml. of water, 5 ml. of 10 per cent. trichloroacetic acid and 20 ml. of absolute alcohol were added, and filtration commenced about half-an-hour later. To D, 20 ml. of Almen's tannic acid solution were added, the mixture filtered after standing about half-an-hour, and the precipitate washed with water.

In each case the complete filtrate and washings were collected for nitrogen determination by Kjeldahl's method. The duplicate non-protein-nitrogen results, which appear in Table I, are typical of others similarly obtained, and demonstrate

TABLE I.

Milligrams non-protein-nitrogen per 100 ml. milk.			
A 45.2	B 36.3	C 36.8	D 30.8
41.0	36.3	37.8	31.6

the following points: Trichloroacetic acid in the cold gave distinctly high and irregular N.P.N. figures. The use of alcohol with more dilute acid gave rather lower but still irregular N.P.N. figures. In both these cases besides the irregular figures, the opalescent filtrates and washings were unsatisfactory. By warming the trichloroacetic acid mixture, clear filtrates and very good agreement of duplicates were always obtained. Still lower N.P.N. figures were regularly obtained by the use of tannic acid (see also Table II).

Further experiments were carried out upon the filtrate obtained after removing the casein from 250 ml. of milk by the isoelectric method previously described. In order to obtain a clear liquid, the mixture was filtered only after some hours, or next morning, so that the finest casein particles could accumulate upon the larger ones.

Portions of 50 ml. of the filtrate were carefully pipetted into small beakers for removal of the soluble protein by various treatments. Usually after precipitation of the protein, the filtrate therefrom was collected in a beaker in which it was boiled for a few seconds. The appearance of a further precipitate showed incomplete removal of protein, so that the necessity for completing the washing and nitrogen determination of the original precipitate could be eliminated. In using trichloroacetic acid, sufficient 20 per cent. or 50 per cent. solution was added to make the final concentration 4 per cent. Lower concentrations (2.5 per cent.) sometimes gave slightly low results, while higher concentrations were avoided because of the work of Hiller and Van Slyke,<sup>9</sup> which indicated that heating in the presence of strengths of 5 per cent. and over was liable to cause hydrolysis. Two tannic acid solutions were tried—A, Almen's, consisting of 240 ml. of 50 per cent. alcohol together with 10 ml. of 25 per cent. acetic acid and 5 grms. of nitrogen-free tannic acid; B, consisting of 10 grms. of tannic acid, 2.5 grms. of sodium acetate, 2.5 grms. of sodium chloride, and 5 ml. of glacial acetic acid, made up to 200 ml. with water. For the 50 ml. portions of filtrate, 10 ml. of A were used or 5 ml. of B.

Trials were made to see if the heat coagulation method could be improved by repeated heating of the protein solution at intervals of some hours, accompanied



by replacement of the acetic acid volatilised by the heat. In other trials, when the protein was precipitated by heating on the water-bath with sufficient magnesium sulphate or sodium sulphate to saturate the cold solution, washing with water was found to dissolve some of the precipitate. This occurred even after repeated heating of the saturated solutions in the presence of sufficient acetic acid; consequently, washing had to be carried out with saturated salt solution. Filtration and washing required to be completed as quickly as possible, a pleated filter being used to facilitate the operation. After rinsing out the beaker two or three times on to the filter, washing could be completed with small amounts of solution if this were allowed to drop from a pipette round the margin of the paper. Alternatively, by means of a small wash-bottle of saturated solution, the filter could be sprayed with a fine stream. After each application the paper should be allowed to drain well, but not for so long that evaporation causes crystallisation to occur. To avoid this difficulty, watch glasses may be used to cover the filter funnels.

Attempts were made to use trichloroacetic acid for the purpose of rendering less soluble the precipitate from saturated salt solutions. These attempts were unsuccessful, partly because in such solutions the protein floated on the top and could not be reached by the dissolved acid. Provided the solutions were not over-heated, the results differed little from those obtained similarly without the use of trichloroacetic acid.

TABLE II.

Expt. No.	Repeated heating with acetic acid only.	4 Per cent. $\text{CCl}_3\text{COOH}$ .		Saturated $\text{MgSO}_4$ and acetic acid.	Saturated $\text{Na}_2\text{SO}_4$ and acetic acid.	4 Per cent. $\text{CCl}_3\text{COOH}$ .		Cold 4 per cent. $\text{CCl}_3\text{COOH}$ .	Tannic acid A	Tannic acid B
		added before heating.	added after heating.			with saturated $\text{MgSO}_4$ .	with saturated $\text{Na}_2\text{SO}_4$ .			
1	7.4	10.5	10.2*	10.7	9.8*	11.4	11.2	Precipitation incomplete*	11.5	11.2
	7.4	10.4	10.2*	10.7	10.0*	11.3	11.0		11.1	11.2
2		11.4	11.5	11.7	11.6	11.4	11.6		11.9	11.8
		11.5	11.4	11.5	11.5		11.7		11.9	11.8
3		8.2	8.1*	8.5	8.4	8.5	8.5	6.7*	8.7	8.5
		8.2	8.1*	8.4	8.3*	8.4	8.5		8.6	8.5

The figures are mg. of nitrogen precipitated from each 50 ml. portion of solution used.

\* In these cases opalescence appeared either in the filtrate after boiling or in the washings.

The results of three separate comparative experiments are given in Table II, and these are typical of others similarly obtained. It is remarkable that, in the case of Experiment 1, repeated heating of the protein solution with acetic acid by itself should precipitate only about 70 per cent. of the protein and yet leave a filtrate which remained clear even on further boiling. Heating of the solution with trichloroacetic acid gives precipitates which filter rapidly, and can be easily washed (with about 1 per cent. acid). If this acid is added after heating, the results may sometimes be very slightly low; whilst if the heating is omitted, precipitation is incomplete at low concentrations.

The use of saturated salt solutions (with or without trichloroacetic acid) regularly gave results which were slightly higher than those obtained by using trichloroacetic acid alone. Various reasons can be suggested for this difference. First, the difficulty of washing the gelatinous precipitate with a solution which tends to crystallise on the filter paper may make it impossible to remove all the non-protein material. Second, the experiments of Wasteneys and Borsook<sup>8</sup> suggest that the saturated salt solution may precipitate any traces of "proteose" material which may be present in milk, but which may not be precipitated by trichloroacetic acid. Third, slight hydrolysis of the protein may be initiated during the heating with the trichloroacetic acid. The last possibility is suggested by some results of Hiller and Van Slyke;<sup>9</sup> but, on the other hand, the action of trichloroacetic acid in rendering proteins insoluble has been emphasised by the experiments described by Loeb.<sup>11</sup>

A number of reagents for the removal of the total protein from milk were studied by Simon,<sup>2</sup> who favoured especially tannic or phosphotungstic acid. A careful study of his figures leads to the conclusion that in the majority of cases tannic acid precipitated slightly more nitrogen than trichloroacetic acid, whilst phosphotungstic acid precipitated more than tannic acid. This comparison of tannic and phosphotungstic acids has been confirmed by the results (Tables I and VII) of Grimmer, Kurtenacker and Berg<sup>12</sup> upon milk sera, while my experiments have emphasised the differences in the amounts of material removed from milk serum by tannic and trichloroacetic acids. Since these experiments were carried out with uranyl acetate (10 ml. of 1.57 per cent. per 10 ml. of milk, diluted to 50 ml.) has been recommended by Kopatschek<sup>13</sup> for the determination of the non-protein-nitrogen content of milk, but I have not yet been able to compare it with other reagents.

As a result of my experience I prefer trichloroacetic acid for the purpose of removing albumin and globulin from milk sera; but, before making a final decision, experiments ought to be carried out to determine the action of trichloroacetic acid upon pure solutions of the milk proteins. In the meantime, the following method is suggested:

**PROPOSED TENTATIVE METHOD.**—To the filtrate obtained after the isoelectric precipitation of the casein, sufficient trichloroacetic acid is added to make the final concentration approximately 4 per cent. The mixture is heated for half-an-hour on the boiling water-bath, and, after standing to cool, it is filtered and washed with a 1 per cent. solution of trichloroacetic acid. The nitrogen content of the precipitate is estimated by Kjeldahl's method.

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## B. THE SEPARATE DETERMINATION OF ALBUMIN AND GLOBULIN.

The accurate analytical separation of the two soluble milk proteins is practically the same problem as the determination of the albumin and globulin of blood serum. The difficulty of this problem is obvious to anyone who reads the extensive literature thereon.

Howe<sup>1</sup> has critically reviewed the various methods which have been proposed for the estimation of serum proteins and has made the statement that "until independent procedures are devised for the identification of the proteins, 'salting out' must remain as the point of departure for future work." This agrees with statements made by Cohn<sup>2</sup> and Schryver.<sup>3</sup> Methods involving the use of the refractometer or viscometer seem to be less satisfactory than "salting out," associated with either gravimetric or Kjeldahl determinations.<sup>4,5</sup>

In deciding what procedure should be adopted for any particular investigation, consideration must be given to the object in view. For certain purposes the methods which Howe<sup>6</sup> has applied to colostrum would provide a series of comparative values of the same degree of accuracy throughout. Such methods are liable to a slight constant error, due to the volume of the precipitate, a fact which Howe apparently realised (p. 65, *loc. cit.*). He worked with colostrum from which the fat had been removed, so that the application of his methods to milk or colostrum containing the fat would magnify the error, since the fat would be precipitated with the protein. The error might be eliminated by washing the precipitate and determining the nitrogen content of the filtrate and washings, instead of using an aliquot part only of the filtrate. Alternatively, duplicates might be precipitated at different dilutions so that a calculation could be made to discover the error due to determinations on aliquot portions of the filtrates. Thus more accurate absolute values for the albumin and globulin content of milk ought to be obtainable. At the same time, one must remember the limitations of fractional precipitation, which are obvious from the statement of Schryver (*loc. cit.*) and the experiments of Woodman.<sup>7</sup>

In dealing with colostrum Howe<sup>6</sup> was able to sub-divide the globulin into no less than three fractions, but the amount of globulin in milk is rather small for this to be attempted, and the validity of such a sub-division has recently been questioned.

I have determined the globulin content of milk by different macro-methods. The filtrate from the isoelectric precipitation of casein is first neutralised to phenolphthalein with *N*/10 sodium hydroxide solution. It is then saturated with an approximately weighed amount of either magnesium or sodium sulphate (anhydrous), which must be stirred in while the temperature is maintained at about 20° C. by means of a water-bath. After standing some time, filtration and washing (with saturated salt solution) ought to be completed without interruption, hence it is preferable not to commence filtering the large bulk of solution until next morning, using a pleated filter. Mechanical impurities are sometimes present in the large amounts of salt required for saturation, so that after washing is completed it may be desirable to wash the globulin through the filter paper into the Kjeldahl flask, with the use of distilled water or a very dilute solution of sodium chloride.

Alternatively, casein and globulin may be precipitated together as in the difference method outlined below. Since considerable dilution of the protein solution is desirable, 10 ml. of milk (weighed as for casein) should be neutralised and mixed with at least 90 ml. of saturated sodium or magnesium sulphate solution, and sufficient extra salt added to saturate 10 ml. of water. The protein precipitate is filtered off, washed with saturated salt solution, and its nitrogen content determined by Kjeldahl's method. In each of the above cases the albumin remaining in the filtrate of saturated salt solution may be separated by acidifying with 3 ml. of 10 per cent. of acetic acid and heating on the boiling water-bath for at least half-an-hour. After cooling, the precipitate is filtered off and washed, preferably with saturated salt solution. The precipitation beaker is rinsed into the Kjeldahl flask with the aid of a little soda, as suggested for casein. Gentle heating during the early stages of digestion is essential to avoid frothing. In preliminary trials with the above methods, the writer obtained the following values from different samples of milk: 0.020, 0.015, 0.027, 0.030, 0.022, 0.0215 grm. of globulin nitrogen per 100 ml. of milk. The figures in each case are the average of several determinations, and, although the agreement between duplicates left a little to be desired, the results are worth recording for comparison with the very few others which have been published. Simon<sup>9</sup> found slightly higher values: 0.051, 0.049, 0.029, 0.043, 0.029, 0.030, 0.034 grm. of globulin nitrogen per 100 grms. of milk. During a study of colostrum, Mrozek and Schlag<sup>10</sup> determined globulin by two different methods, but the figures differ so widely that their reliability may be questioned. At the forty-second milking, when the colostrous phase ought to have passed off, they found globulin nitrogen 0.12 per cent. by one method and 0.02 per cent. by another.

**CONCLUSIONS.**—On the basis of the foregoing investigations, the following scheme may be suggested for the purpose of obtaining values for casein, albumin and globulin in milk.

A. Casein by isoelectric precipitation at *pH* 4.6.

B. Casein and globulin by neutral saturated magnesium sulphate or sodium sulphate.

C. Total protein by warm 4 per cent. trichloroacetic acid.

From B—A globulin may be obtained; from C—B albumin may be obtained. Alternatively, albumin and globulin may be determined by treating the filtrate from A with trichloroacetic acid, and albumin obtained by subtracting the globulin value, B—A. In order to avoid the difficulties encountered in dealing with large volumes of saturated solutions, which so readily crystallise on the paper during filtration, a micro-procedure (similar to that of Howe), together with the micro-Kjeldahl method for nitrogen, may be found advantageous.

This paper represents part of the work carried out during my tenure of the Pedler Research Scholarship of the Institute of Chemistry, the assistance of which and the interest of the members of the Pedler Fund Committee are gratefully acknowledged. I wish to add my appreciation of the facilities made available to me at the National Institute for Research in Dairying, and especially of the valuable advice of Capt. J. Golding, Head of the Chemical Department.

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#### DISCUSSION.

The PRESIDENT said that the Society was glad to have the opportunity of publishing the work of the Pedler Scholar of the Institute of Chemistry, and was very grateful to Captain Golding for his synopsis of the four papers.

Mr. BACHARACH said that by a strange coincidence he had that morning, while anticipating the pleasure of hearing Dr. Moir's papers, been confronted with a paper, 56 pages long, by two German chemists whose work touched Dr. Moir's very closely. They were investigating the residual nitrogen of milk, and set out to revise the existing methods of separating casein, albumin and globulin. Although he had not yet been able to read this paper thoroughly, there were one or two very interesting points he had noticed. The first was that, although these authors did not use the sodium acetate buffer solution, they considered the only safe method of determining casein and nitrogen in milk to be iso-electric precipitation with acetic acid at pH 4.6; they had overlooked the advantage of using a buffer with sour samples. They tried other methods, using tannic acid and phosphotungstic acid. They stated that globulin was present in normal milks to a very small extent; this was in disagreement with Dr. Moir, who found quite appreciable quantities of globulin in normal milks. It was to be hoped that Dr. Moir would continue his investigations of the various nitrogenous constituents of

cow's milk; when he had done that, he might, perhaps, turn his attention to a problem even harder than this—the precise distribution of nitrogen in human milk.

Mr. J. H. BUSHILL referred to the two methods adopted to prove the similarity of the casein precipitated at  $pH$  4.6 by an acetate buffer solution, and that precipitated at  $pH$  4.2 by the usual acetic acid method. He doubted whether these methods would detect the presence of 1 to 2 per cent. of lactalbumin or of lactoglobulin in the casein, and asked Captain Golding whether he had any similar curves showing the effect of the addition of such an amount of lactalbumin or of lactoglobulin to a casein precipitate.

Captain GOLDING said that he was sure Dr. Moir would be glad to see the German paper referred to by Mr. Bacharach. There were very many contrary suggestions made on this subject. Dr. Moir determined the amount of non-protein nitrogen in milk; if the amount of globulin in milk was small the amount of non-protein nitrogen must be similar. Captain Golding pointed out that he had not been able to read the whole of the papers to the meeting, but with regard to the curves Dr. Moir had gone into the matter fully. Precipitation with acid only gave a figure about 2 per cent. too low, and so he took it that the precipitation with acetic acid by itself would give a substance which had been generally regarded as casein. He was then getting a substance which was, in fact, pure casein, or casein plus a little albumin and globulin, and went into that point and proved that this method would show contamination with 2 per cent. of albumin.

The following communication has been received from Dr. MOIR:

When my papers were discussed at the Society's meeting, on November 5th, 1930, a speaker enquired about the curves which albumin and globulin would give with the formol titration or the hypobromite oxidation methods. In the time at my disposal I was unable to investigate this matter. A study of the work I cited by Woodman, Abderhalden and Kroner, and by Goldschmidt and Steigerwald, would lead one to expect that, owing to their structural differences, albumin and globulin would give distinctly different curves from casein. Assuming, for example, that the higher nitrogen values obtained at  $pH$  4.6 were due to a precipitate of (say) casein plus albumin, instead of (say) casein only at  $pH$  4.2, the curves from the former ought to differ slightly, but definitely, in shape from those of the latter.

Another method which I tried was the determination of the ratio of nitrogen to sulphur in casein, which has a very low sulphur content compared with that of lactalbumin. After overcoming a number of difficulties, I obtained promising results, but unfortunately was unable to complete them to the stage required for publication. Apart from these experimental methods of investigating the casein precipitated at different  $pH$  values, I feel that the new method I have evolved derives very great support from the fundamental conceptions associated with the iso-electric point, which is one of the most definite physico-chemical properties of a protein.

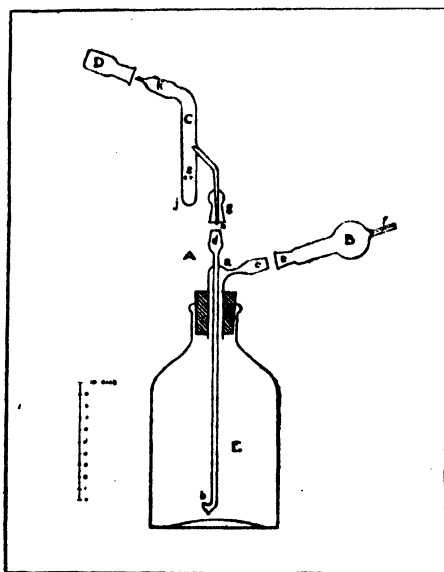
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## A Storage and Delivery Apparatus for Antimony Chloride Solution and other Corrosive Reagents.

By G. MIDDLETON, B.Sc., A.I.C.

(Read at the Meeting, December 3, 1930.)

Of the various colour tests which have been proposed for cod-liver oil, that of Carr and Price (*Biochem. J.*, 1926, 20, 497) has found most general adoption, on account of its quantitative character. The chromogen reaction, of which it is a measure, is employed as an indication of the vitamin A content of the oil. The reagent (a solution of antimony chloride in chloroform), is volatile, poisonous and corrosive, and, when handling it, special precautions have to be observed—in particular against contact with the fingers, and against unnecessary exposure to moist air.



In the routine examination of oils, the need for a convenient way of storing and measuring the reagent was keenly felt. To meet this need a special apparatus has been designed, and it is thought that other workers may find the following description useful.

The purpose of the special joints at *g* and *k* is to prevent the reagent from getting on to the ground-glass surfaces and cementing them together by the formation of antimony oxychloride.

**DESCRIPTION OF APPARATUS.**—The apparatus, which is shown in section in the figure, consists of six portions:

- (1) An amber glass bottle, *E*, conveniently holding about 600 c.c.
- (2) A glass portion, *A*, fitted to the bottle, *E*, by means of an ordinary cork, and consisting of an internal joint, *a*, with the inner tube, *ab*, extended downwards into the liquid at *b*, the end, *b*, being turned over through an angle of  $180^\circ$ , as shown, in order to draw up the reagent without disturbing the sediment. At the lowest point of this end there is made a very small hole, the object of which is to allow the escape of any of the heavy oily liquid which separates from the reagent on keeping, and which would otherwise accumulate in the lower bend of the tube and be carried up into the measuring pipette. At *c* and *d* are the inner portions of ground-glass joints.
- (3) A calcium chloride tube, *B*, with ground joint, *e*, fitting on to *c*. The other end, *f*, is open but constricted.
- (4) A rubber bulb fitting on to *f*.
- (5) The measuring portion, *C*, with ground-glass joint, *g*, fitting outside the taper *d* of *A*, having an inner tube, *h*, the end of which is cut at an angle in such a way that when the joint is assembled, the lowest part nearly touches the wall of *A*, so that liquid drains straight down from *h* to *a*. The portion, *j*, is of such capacity that the measuring apparatus delivers exactly 2 c.c. Owing to the transient nature of the colour produced in the reaction, and the consequent necessity for quick working, not more than one or two seconds should be allowed in the calibration for drainage. At *k* there is a ground taper running off into the jet which is ground off at an angle.
- (6) A glass cap, *D*, ground on to the taper *k* of *C* in such a way that it does not come into contact with the jet.

The scale represents divisions of cm. in a total of 10 cm.

**USE OF THE APPARATUS.**—The tube, *B*, is filled with calcium chloride and the bottle, *E*, with reagent.\* When required for use, the cap, *D*, is removed, and the measuring portion, *C*, filled by blowing air (cautiously at first) through *B* by means of the rubber bulb. The excess of liquid in *C* runs back on releasing the pressure, but one or two minutes are allowed so that the tube, *h*, may drain thoroughly. *C* is then detached, care being taken that none of the liquid in it runs back through the tube, *h*, and the 2 c.c. of reagent are poured out. When not in use, the cap, *D*, should be replaced and the rubber bulb removed from *f*. The reagent should not be allowed to get on the ground parts of the joints, as it may cause them to stick together, in which case they should be freed by soaking in hydrochloric acid.

The special features claimed for this apparatus are as follows: (1) The reagent has a minimum amount of exposure to moist air. (2) There is no danger of accidental contact of the reagent with the hands of the operator. (3) The sediment in the bottle is not disturbed. (4) The measuring portion is always ready for use and does not need washing out and drying after each time it is used, as with an ordinary dipping pipette. (5) There are no ground-glass taps or joints in actual contact with the reagent.

The apparatus as described above has been in continuous use in the laboratories of The British Drug Houses, Ltd., for several months, and has been found most advantageous. It may be used also for other corrosive liquids, *e.g.* bromine, in which case the cork must be replaced by a ground joint fitting in the neck of the bottle; and especially for liquids, such as strongly alkaline solutions, which cannot be used with glass taps.

\* It is recommended that the apparatus should first be given a trial with chloroform only.



## Tests for Impurities in Ether.

### Parts II. and III.

BY G. MIDDLETON, B.Sc., A.I.C., AND F. C. HYMAS, B.Sc., A.I.C.

*(Read at the Meeting, December 3, 1930.)*

#### PART II: TESTS FOR ACETALDEHYDE.

ACETALDEHYDE and peroxide form the two impurities of most significance in ether, not only because they are the cause of undesirable after-effects when the ether is used for the production of anaesthesia, but also because of the intimate relation between the two. On the one hand, the presence of acetaldehyde increases the rate of formation of peroxide in ether, and, on the other, the organic peroxide itself decomposes, forming acetaldehyde, so that the rate of oxidation increases progressively. The importance of a delicate test, so that a high standard of freedom from acetaldehyde may be maintained, is therefore evident.

The current pharmacopoeias all require anaesthetic ether to comply with tests for freedom from aldehyde; the French Codex also requires freedom from vinyl alcohol. If, as is often considered, the latter is a desmotropic form of acetaldehyde,<sup>2</sup> the two forms should be in equilibrium, and there would be no object in testing for each separately.

Vinyl alcohol has never been obtained in the pure state, and the evidence for its existence is based on Poleck and Thummel's<sup>3</sup> examination of the precipitate which they obtained on treating impure ether with a solution of mercuric chloride and potassium bicarbonate. This precipitate is not obtained with acetaldehyde, so that the two compounds cannot, without further evidence, be regarded as desmotropic. It is proposed for this reason to deal separately with tests for acetaldehyde and for the isomeric vinyl alcohol, the latter being considered together with the tests for other vinyl compounds.

**DETERMINATION OF SENSITIVENESS.**—The sensitiveness of a test for acetaldehyde may be expressed in terms of the smallest proportion of acetaldehyde required to give a just distinctly perceptible reaction. Except where otherwise stated, all tests were carried out in colourless glass-stoppered bottles of about 30 c.c. capacity (1 ounce bottles), using 25 c.c. of ether and 6 to 8 c.c. of the reagent. A standard acetaldehyde solution was prepared by dissolving about 5 c.c. of freshly distilled acetaldehyde in 100 c.c. of pure aldehyde-free ether, the strength of this solution being determined by adding 5 c.c. to an excess of a neutral solution of hydroxylamine hydrochloride in a stoppered bottle, shaking well, and titrating the liberated acid with normal alkali, using methyl orange as indicator. The solution was then diluted with aldehyde-free ether to the required strength. Both the pure ether and the acetaldehyde solution were freshly prepared as required, and control experiments were carried out in all cases, in order to ensure that the pure ether gave no reaction in the test under investigation.

The detection of aldehyde is complicated by the fact that ether peroxide also gives positive reactions with reagents for acetaldehyde. For this reason the sensitiveness of tests towards ether peroxide was also determined, using the method given in Part I of this paper.<sup>1</sup> The results obtained are given below:

Test.	Sensitiveness	
	to acetaldehyde (parts per million).	to ether peroxide (cal- culated as $H_2O_2$ , in parts per million).
Ammoniacal silver nitrate .. ..	1000	over 560
Solid potassium hydroxide (6 hours) ..	40	over 560
Diazobenzene sulphonic acid .. ..	3	—
Schiff's reagent, with pyrogallol .. ..	2	0.2
Tollens' reagent .. ..	1	9
Nessler's reagent .. ..	1	5
Nessler's reagent, diluted 1-4 .. ..	1	2.5

**THE CAUSTIC ALKALI TEST.**—In all modern pharmacopoeias, with only one exception, either sodium or potassium hydroxide is used as a test for the presence of aldehyde in ether. The prescribed time of contact varies from one-half to six hours, and there is no uniformity in the instructions concerning exposure to light during this period.

This test has been fully investigated by Mallinckrodt,<sup>4</sup> who found that the official test of the United States Pharmacopoeia with solution of potassium hydroxide had a sensitiveness of 300 parts per million under optimum conditions. The official German test with solid caustic potash was found to be more sensitive, but not capable of detecting less than 100 parts per million with certainty.

Though this very low sensitiveness, compared with that of other tests available, is in itself sufficient to justify the abandonment of this test, the significance it has acquired on account of its almost universal official adoption calls for a more detailed examination.

When freshly broken potassium hydroxide is added to ether containing acetaldehyde, it is observed that, after standing for some time, a brown colour is produced at certain points on the surface of the potash. The colour appears on the original outer surfaces of the sticks of alkali, owing to the fact that a certain amount of moisture is necessary for the development of the colour. In the absence of moisture, ether containing as much as 750 parts of acetaldehyde per million gives no colour after three hours' exposure.

The presence of alcohol in the ether under examination often causes the formation of a turbidity, and, on long standing, a yellow colour. This interference is removed to some extent by the addition of water.

Our experience is in general agreement with the above results, taken direct from the paper of Mallinckrodt, who sums up the test in the following words: "Anyone using the test with solid potassium hydroxide day in and day out on ethers of varying composition will be struck by the variations in the phenomena

attending the test, and herein is its chief defect; for the observer must make allowances for the disturbing influences caused by the presence in the ether of such common impurities as alcohol and peroxides. The pure brown coloration on the lumps of potash normally produced by pure aldehyde in pure ether may be obscured or replaced more or less by the other appearances which, while difficult to describe in an adequate manner, are readily recognised after having once been seen."

**SCHIFF'S REAGENT.**—This is employed as an official test for aldehyde only in the French Codex (1908), which requires that ether shall not redden a solution of rosaniline decolorised with bisulphite. No time limit is given for the test, although this is very important, as with small quantities of aldehyde the colour develops very slowly, whilst the purest ether obtainable will give a colour with the reagent on long standing. Further, the colour is more or less intense according to the amount of shaking, and depends to some extent on the temperature. In spite of these objections, the test is a very useful one if employed under standard conditions, as it combines sensitiveness with specificity for aldehyde. Our procedure is to shake the ether thoroughly with the reagent, allow the mixture to stand for 10 minutes at room temperature, and note the colour of the lower layer. Only the purest samples of ether show no colour after this time.

All attempts to prepare ether that would give no colour on standing for a long time with Schiff's reagent proved unsuccessful, and it was finally found that the progressive development of colour was due to the formation of aldehyde from the ether under the conditions of the test, and that sulphur dioxide greatly increases the velocity of oxidation of ether in air. This effect may be inhibited by the addition of pyrogallol, which exerts an anti-oxidising action. By the addition of 0.1 per cent. of pyrogallol to Schiff's reagent the sensitiveness to aldehyde is not decreased, while at the same time no colour is obtained even after several hours' contact with pure ether. In the presence of aldehyde the maximum colour is produced in about 20 minutes.

The Schiff's reagent was prepared as described in the British Pharmacopoeia, with the use of fuchsin known to be sensitive. Samples of old and badly deteriorated ether, containing a large amount of peroxide, give a very weak reaction with Schiff's reagent; but, after dilution with 20 parts of pure ether, they then gave a strong reaction with the reagent. A strong reaction was also obtained after the ether had been washed with four successive small portions of water, in order to remove hydrogen peroxide; and it was found further that the presence of hydrogen peroxide prevented the development of colour with Schiff's reagent. It follows, therefore, that in an ether, giving a heavy reaction for peroxides, the absence of a colour with Schiff's reagent does not indicate the absence of aldehyde.

**AMMONIACAL SILVER NITRATE.**—In the French Codex, a solution of silver nitrate, to which ammonia is added until the precipitate has almost redissolved, is given as an additional test for aldehyde; but no statement of the exact time of

contact or of quantities of ether and solution to be taken is made. The figure of 1000 parts per million, given above as representing the sensitiveness, refers to a period of 20 minutes. With smaller proportions of aldehyde a positive reaction is obtained only after standing for several hours. It is, however, unnecessary to consider this test in further detail, as an ammoniacal silver solution in the form of Tollens' reagent is very much more sensitive and more rapid in its indications.

**TOLLENS' REAGENT.**—The reagent was prepared and employed as described in Part I of this paper.<sup>1</sup> The test mixtures were kept in the dark, and the full colour developed within 10 minutes. The test is not specific for aldehyde, as it is given also by peroxide.

**p-DIAZOBENZENE-SULPHONIC ACID.**—Though this reagent is frequently used for the detection of acetaldehyde, its employment for testing ether does not appear to have been suggested; a closer examination of the test, with this object in view, was, therefore, thought desirable. The test was applied as follows:—To 2 c.c. of a 1 per cent. solution of sulphanilic acid in 5 per cent. hydrochloric acid were added 2 c.c. of 0.38 per cent. sodium nitrite solution. After a few moments 25 c.c. of the ether and about 0.3 grm. of solid caustic soda were added, followed by a piece of semi-solid sodium amalgam about the size of a pea. In presence of aldehyde a red colour developed slowly in the aqueous layer. Though at first this test appeared promising, it was found that pure aldehyde-free ether gave a red colour with the reagent, even when the amount present was only sufficient to give a layer of ether 1 mm. deep above the reagent. Further investigation showed that alcohol also gave a red colour. One mgrm. of specially purified aldehyde-free alcohol gave a distinct red colour, possibly due to the rapid oxidation of alcohol to aldehyde, which occurs on exposure to air of strongly alkaline spirit solutions. Half a mgrm. of acetaldehyde gave a red colour with 4 c.c. of the reagent, but 0.1 mgrm. and smaller amounts gave a brown colour which was not so distinct, in view of the brownish-yellow colour obtained in the absence of aldehyde.

**NESSLER'S REAGENT.**—Most commercial samples of ether give at least a yellow turbidity with this reagent, and Baskerville and Hamer<sup>5</sup> state that it is impossible to obtain ether which gives an entirely negative reaction. This is not correct; ether giving no reaction with Nessler's reagent may be prepared experimentally.

The British Pharmacopoeia method of preparing Nessler's reagent was employed, and the solution was tested for sensitiveness before use. When 25 c.c. of ether containing a trace of acetaldehyde are treated with 5 c.c. of the reagent, a brownish-red colour develops in the aqueous layer in about half a minute, changing in a few minutes to grey. Though the latter tint is slightly less conspicuous, it has the advantage, over the transient brown colour, of greater permanence. If acetone is added to a sample of ether which gives no colour or precipitate with the reagent, a cream-coloured turbidity or precipitate is obtained, quite distinct from that due to acetaldehyde or ammonia, but liable, if sufficiently heavy, to obscure the reaction of the last two. The test is very sensitive to acetone.

The German and Belgian Pharmacopoeias allow narcotic ether to give an opalescence, but no turbidity, with Nessler's reagent. In the Dutch and Swedish official tests the reagent is diluted with water before use. Comparative tests showed that there was no difference in the sensitiveness of diluted and undiluted reagent towards either acetone or acetaldehyde, but that the diluted reagent was slightly more sensitive towards peroxide. The diluted reagent has the advantage that the yellow colour due to the reagent itself is greatly reduced. It is, therefore, recommended that, in employing this test, 25 c.c. of the ether should be shaken well with a mixture of 1 c.c. of Nessler's solution and 4 c.c. of water, and allowed to stand for 15 minutes.

**PHARMACOPOEIAL TESTS.**—A table of the official tests for acetaldehyde adopted in the various pharmacopoeias is given. It shows that chief reliance is placed on the caustic alkali test, which has been shown to be insensitive and unreliable.

Pharmacopoeia.			Ammoniacal silver nitrate.	Nessler's reagent.	Caustic alkali.	Schiff's reagent.
British, 1885	..	..	—	—	—	—
1898	..	..	—	—	+	—
1914	..	..	—	—	+	—
American, 10, 1926	..	..	—	—	+	—
French, 1908	..	..	+	—	+	+
German, 6, 1926	..	..	—	+	+	—
Belgian, 3, 1906	..	..	—	+	+	—
Swedish, 10, 1925	..	..	—	+	+	—
Norwegian, 4, 1913	..	..	—	—	+	—
Italian, 4, 1920	..	..	—	—	+	—
Swiss, 5, 1926	..	..	—	—	+	—
Dutch, 5, 1926	..	..	—	+	—	—
Spanish, 7, 1915	..	..	—	—	+	—
Portuguese, 1876	..	..	—	—	—	—
Japanese, 4, 1922	..	..	—	—	+	—
Argentine, 1921	..	..	—	—	+	—
Danish, 1907	..	..	—	—	+	—
Roumanian, 1926	..	..	—	—	+	—

+ indicates that the test is adopted.

— indicates that the test does not appear.

**DISCUSSION.**—It has been shown that the caustic alkali test, which is in general use, is not sufficiently delicate to ensure the freedom of anaesthetic ether from objectionable quantities of acetaldehyde. This test should, therefore, be superseded by a more sensitive test.

Of the three more sensitive reagents given in the table, both Tollens' and Nessler's reagents give positive reactions with other impurities which may be present in ether, so that the interpretation of the results obtained is uncertain.

The application of Schiff's reagent has been limited by the uncertainty as to its significance, owing to the colour showing a progressive increase with time. This objection has now been removed by the addition of pyrogallol to the reagent,

to inhibit the catalytic effect of the sulphur dioxide, and the test may be recommended for general and official adoption.

### PART III: TESTS FOR ACETONE.

Only two pharmacopoeias employ tests specific for acetone, and ether containing it may fail to pass the tests of two others.

Baskerville and Hamer<sup>6</sup> state that they "cannot anticipate its presence in ether prepared from pure ethyl alcohol," and that "it is unnecessary to look for such an improbable contaminant at the present time." This view is by no means justified; many commercial samples of high grade narcotic ether contain traces of acetone, whether they have been made from denatured or from duty-paid spirit.

**DETERMINATION OF SENSITIVENESS.**—For determining the sensitiveness of the different tests, acetone prepared from the sodium iodide compound was used as a standard and dissolved in acetone-free ether. The results obtained are given below. In all cases 25 c.c. of ether were taken for the test.

Test.	Sensitiveness, etc., in parts per million.
Mercuric chloride and baryta .. .. .	100
Hydroxylamine and hypochlorite <sup>6</sup> .. .. .	100
Sodium nitroprusside (German Pharmacopoeia)	25
Sodium nitroprusside (B.P.C.) .. .. .	20
Acid mercuric sulphate .. .. .	5
Vanillin (Dutch Pharmacopoeia) .. .. .	1
Nessler's reagent .. .. .	0.5 (upper limit 20)

**MERCURIC CHLORIDE AND BARYTA.**—Twenty-five c.c. of ether are shaken with 5 c.c. of saturated barium hydroxide solution and 5 drops of mercuric chloride solution. The aqueous layer is run off, filtered, and the filtrate tested for mercury by the addition of several drops of ammonium sulphide solution. This test was employed in the Dutch Pharmacopoeia of 1905, but in a less sensitive form—only 2 c.c. of ether being taken.

**SODIUM NITROPRUSSIDE.**—There is little to choose between the two forms of the test which were examined, but that of the German Pharmacopoeia has the advantage that the colour due to the reagent itself is less than is the case with the test of the British Pharmaceutical Codex.

**ACID MERCURIC SULPHATE.**—Twenty-five c.c. of ether are shaken with 5 c.c. of water, the aqueous layer separated, mixed with 1 c.c. of Denigès' acid mercuric sulphate reagent, and heated for half-an-hour in the water bath. If acetone is present, a white precipitate is obtained.

**VANILLIN.**—The test of the 1926 Dutch Pharmacopoeia is as follows:—Twenty c.c. of ether are shaken with 5 c.c. of water, the water layer is separated, and to it are added 5 mgrms. of vanillin and a piece (about 0.75 grm.) of potassium hydroxide. The mixture is warmed (cautiously at first) for 15 minutes at 60 to 70° C., without shaking. The presence of acetone is indicated by an orange or red layer above

the potash. This is useful as a specific test for acetone when the presence of other impurities prohibits the use of Nessler's solution for this purpose.

**NESSLER'S REAGENT.**—Though Nessler's reagent was found to be a very sensitive test for the detection of small quantities of acetone, it was also found that a positive reaction was no longer obtained when the concentration of the acetone exceeded a certain limit, which, for the conditions employed, was 20 parts per million. When the concentration is higher than this a transient precipitate may be obtained on the addition of the ether to the reagent, and a precipitate may also be formed on long standing, but the cream-coloured turbidity or precipitate which is most characteristic of acetone does not appear.

No difference in sensitiveness could be observed between Nessler's solution (prepared according to the British Pharmacopoeia), and the same solution diluted with 5 parts of water, as used by the Dutch and Swedish Pharmacopoeias. The latter form has the advantage that the natural colour of the reagent is considerably reduced.

**PHARMACOPOEIAL TESTS.**—The only pharmacopoeias which employ tests which respond to acetone are the following :

Pharmacopoeia.			Nessler's reagent.	Vanillin.	Sodium nitroprusside.
German, 6, 1926	..	..	+	—	+
Belgian, 3, 1906	..	..	+	—	—
Dutch, 5, 1926	..	..	+	+	—
Swedish, 10, 1925	..	..	+	—	—

Owing to the fact that Nessler's reagent forms a satisfactory test for acetone only when the concentration of the acetone is below a certain limit, it cannot be recommended for general adoption. The Dutch vanillin test should, therefore, replace it.

**SUMMARY.**—(1) A comparison of the sensitiveness of tests for acetaldehyde and acetone is given.

(2) The colour developed with Schiff's reagent, even with the purest ether, is due to rapid oxidation of the ether during the period of the test—this reaction being catalysed by sulphur dioxide. The addition of 0.1 per cent. of pyrogallol to the reagent prevents this, while it does not interfere with the sensitiveness of the reagent towards acetaldehyde.

(3) The caustic alkali test should be superseded by a more delicate test.

(4) Nessler's reagent is a sensitive test for small quantities of acetone, but fails to indicate the presence of large quantities.

(5) The tests recommended for official adoption are: for acetaldehyde, the modified Schiff's reagent; and for acetone, the vanillin test of the Dutch Pharmacopoeia.

We wish to express our thanks to the Directors of The British Drug Houses, Ltd., for permission to publish this paper.

CORRECTION.—In Part I of this communication<sup>1</sup>, page 205, line 19: For "100 c.c. of 3.3 per cent. (w/v) sulphuric acid" read "300 c.c. of 3.3 per cent. (w/v) sulphuric acid."

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## Nickel Uranyl Acetate as a Qualitative Reagent for Sodium.

BY P. FELDSTEIN AND A. M. WARD, D.Sc., Ph.D., A.I.C.

DURING the last few years several papers have been published dealing with the use of certain double uranyl acetates proposed by Streng (*Z. wissenschaft. Mikrosk.*, 1886, **3**, 129; *Chem. Zentrbl.*, 1886, **17**, 488) as microchemical reagents for the sodium ion. Thus a solution containing magnesium uranyl acetate has been investigated by Miholić (*Bull. Acad. Sci. Zagrab.*, 1920, **16**), Blanchetière (*Bull. Soc. Chim.*, 1923, **33**, 807), Kolthoff (*Pharm. Weekblad*, 1923, **60**, 1251), Crepaz (*Ann. chim. applic.*, 1926, **16**, 219), Caley and Foulk (*J. Amer. Chem. Soc.*, 1929, **51**, 1664), Caley (*ibid.*, 1930, **52**, 1349), and Kahane (*Bull. Soc. Chim.*, 1930, [IV], **47**, 382; *J. Pharm. Chim.*, 1930, **11**, 425). Zinc uranyl acetate has been investigated by Kolthoff (*Z. anal. Chem.*, 1927, **70**, 397), Barber and Kolthoff (*J. Amer. Chem. Soc.*, 1928, **50**, 1625; 1929, **51**, 3233), Malitzky and Tubakaiev (*Mikrochem.*, 1929, **7**, 334), Blenkinsop (*J. Agric. Sci.*, 1930, **20**, 51), and cobalt uranyl acetate by Caley (*J. Amer. Chem. Soc.*, 1929, **51**, 1965).

In our experience, zinc uranyl acetate, as proposed by Barber and Kolthoff, is more sensitive than a solution of magnesium uranyl acetate (containing a concentration of uranyl acetate equal to that used in the zinc salt), and gives a more granular precipitate than that obtained with the magnesium salt. The cobalt reagent is less sensitive than either of these, and the large volumes required make it impracticable as an ordinary reagent. Streng also mentioned that other double uranyl acetates give sparingly soluble precipitates of triple acetates containing sodium. We have tried these, and have found that nickel uranyl acetate compares very favourably with the reagents already proposed. It is quite as sensitive



as the zinc reagent. The light green precipitate which separates from the dark green solution of nickel uranyl acetate is rather more granular, and settles more rapidly than the corresponding zinc salt. The crystals, when viewed under the microscope, resemble those of the zinc salt, and appear to be octahedra.

The nickel reagent used was prepared as follows: A mixture of 70 grms. of uranium acetate, 200 grms. of nickel acetate, 60 c.c. of glacial acetic acid, and 940 c.c. of water was warmed and stirred until the solids had dissolved. The solution was allowed to stand at room temperature for several hours, with occasional shaking, and was then filtered through a dry filter into a dry bottle.

**DETECTION OF SODIUM.**—The test was carried out by adding 2 c.c. of the reagent to 0.5 c.c. of sodium chloride solutions of known strengths. With 0.1 per cent. solution of sodium chloride a precipitate forms immediately; with 0.02 per cent. a precipitate is formed, and settles after a few minutes. Potassium chloride gives no precipitates at corresponding concentrations; from solutions of 5 per cent. strength and above, a precipitate rapidly forms (gentle agitation assists the formation of the precipitate in a well-crystallised condition); below these strengths, precipitation becomes much slower. With 2 per cent. potassium chloride solution no precipitate forms at once, but a slight precipitate separates after a few hours. The behaviour with zinc uranyl acetate is analogous, and the precipitate which separates from the solution is described by Barber and Kolthoff (*J. Amer. Chem. Soc.*, 1928, 50, 1630) as zinc potassium uranyl acetate, but Kolthoff (*Z. anal. Chem.*, 1927, 70, 490) refers to it as potassium uranyl acetate. The precipitate obtained from the nickel uranyl acetate, viewed under the microscope, is seen to consist of long fine needles, and is identical in appearance with that obtained from the zinc uranyl acetate solutions. Further, the precipitate obtained from nickel uranyl acetate is yellow, and does not contain nickel; there can be no doubt that it is potassium uranyl acetate, which can be precipitated from moderately concentrated solutions of potassium chloride and uranyl acetate (e.g. 7 per cent. uranyl acetate solution immediately gives a heavy precipitate with 10 per cent. potassium chloride, and this appears identical under the microscope with that obtained in the foregoing cases). The contrast between potassium uranyl acetate and sodium nickel uranyl acetate under the microscope is most striking, and provides a ready means of distinguishing between sodium and potassium. If, however, a microscope is not available, and the presence of potassium has been determined, a rougher test for the presence of sodium can be made as follows: Filter off the precipitate, wash twice with 2 to 3 c.c. of cold methylated spirit, dissolve the residual precipitate in water, add ammonium chloride and ammonium hydroxide, and, without filtering the precipitated ammonium uranate, add dimethylglyoxime. A positive test for nickel shows the presence of sodium.

**INFLUENCE OF POTASSIUM.**—Barber and Kolthoff (*loc. cit.*, p. 1630) have recorded that if potassium ions are present in sufficient amount to cause a precipitate to form, zinc uranyl acetate solution is less sensitive to sodium ions. The following observations on nickel uranyl acetate illustrate this point. A solution

(0.5 c.c.) containing potassium chloride (2.5 per cent.) and sodium chloride (0.02 per cent.) was examined in the usual way. A definite small precipitate was present after 5 minutes. This was examined under the microscope and found to consist of crystals of sodium nickel uranyl acetate only; the amount was insufficient to apply the precipitation reaction for nickel already described. When, however, the concentration of potassium chloride was increased to 7.5 per cent., whereas 0.04 per cent. of sodium chloride gave a precipitate immediately, in which microscopic examination showed sodium nickel uranium acetate definitely to be present, lower concentrations of sodium chloride did not give a conclusive reaction for sodium.

If sodium is present in the solution in relatively high concentration, sodium uranyl acetate may be precipitated as well as the triple salt, for 5 per cent. solutions and stronger of sodium chloride give an immediate precipitate with uranyl acetate (7 per cent.). With a 2 per cent. solution of sodium chloride a precipitate slowly forms; whilst a 1 per cent. solution gives no precipitate during 24 hours.

Nickel uranyl acetate is thus seen to be a very suitable reagent for sodium in the ordinary scheme of qualitative analysis. Usually the only cations present in the solution would be ammonium, magnesium, potassium, and sodium; ammonium and magnesium are without effect on the reagent, and the influence of potassium has been considered. Lithium causes precipitation of a salt closely resembling the sodium triple salt in appearance, and containing lithium, nickel and uranium.

**INFLUENCE OF ANIONS.**—The only common anion likely to be present at this stage which would cause complications is phosphate. The appearance of the very finely divided precipitate of uranyl phosphate is quite different from that of the sodium precipitate. It can be at once distinguished under the microscope from sodium nickel uranyl acetate, or the less sensitive test for sodium may be carried out by filtering off the precipitate, washing twice with 2 to 3 c.c. of methylated spirit, shaking the precipitate with water (uranyl phosphate will remain undissolved), and then adding ammonium chloride and ammonium hydroxide, followed by dimethylglyoxime; the formation of nickel dimethylglyoxime shows the presence of sodium.

We have not attempted to use the reagent for the determination of sodium, as has been done in the cases of zinc and magnesium uranyl acetates. The method, which is rapid, has yielded results of moderate accuracy in the hands of the investigators cited. We hope next to investigate the application of nickel uranyl acetate to this.

**ANALYSIS OF SODIUM NICKEL URANYL ACETATE.**—The salt (0.60 gm.) was dissolved in water (200 c.c.), and the solution treated with 20 grms. of ammonium chloride dissolved in a small amount of water, and then with ammonia in slight excess. The precipitate of ammonium uranate was allowed to settle, washed twice by decantation with ammonium chloride solution, and then dissolved in dilute hydrochloric acid, and precipitation repeated as above. The precipitate was filtered off, well washed with ammonium chloride solution, and ignited in a platinum

crucible to  $U_3O_8$ . Nickel was precipitated from the filtrate by dimethylglyoxime, and the combined filtrates were evaporated to dryness. A small amount of nickel was found to be present in the residue obtained. Most of the ammonium salts were expelled, and the nickel determined by precipitation with dimethylglyoxime. Sodium was determined in the filtrate by evaporation and weighing as sodium chloride. Acetate was determined in a separate sample by distillation with phosphoric acid. Carbon and hydrogen were determined by combustion. The figure for carbon was obtained on the assumption that the material remaining after the combustion of the triple acetate was sodium carbonate, uranium oxide and nickel oxide.

	Found. Per Cent.	Calculated for $NaNi(UO_2)_2(CH_3CO_2)_6 \cdot xH_2O$ .		
		( $x=6$ ). Per Cent.	( $x=6.5$ ). Per Cent.	( $x=9$ ). Per Cent.
$UO_2$	52.80	52.90	52.60	51.10
Ni	3.81	3.85	3.83	3.72
Na	1.49	1.50	1.495	1.45
$CH_3CO_2$	34.3	34.7	34.5	33.5
H	2.55	2.55	2.59	2.84
C	13.98	13.72	13.64	13.25

The formulae given in the papers cited for the compositions of zinc or magnesium sodium uranyl acetates generally contain 6 or 9 molecules of water. Often experimental figures are not given in support. Caley and Foulk (*loc. cit.*) consider, from their results, that the magnesium salt contains  $6.5 H_2O$ . Our experimental results are given, together with the calculated values for 6, 6.5, and 9 of  $H_2O$ . They are not in agreement with the 9  $H_2O$  formula, but we do not regard them as of sufficient accuracy to decide between the formulae containing 6 and 6.5  $H_2O$ .

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## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### THE DIPHENYLAMINE TEST FOR NITRATES IN MILK AS A MEANS OF DETECTING ADDED WATER, AND THE EFFECT OF DRENCHING COWS WITH "NITRE."

THE method of applying this test, described by Lerrigo (ANALYST, 1930, 55, 433), has proved very satisfactory in our hands. So far, no samples of genuine milk have given a positive reaction for nitrates, whilst samples containing added water have given distinct indication of its presence. We find no difficulty in detecting 5 per cent. of added water containing 0.5 part per 100,000 of nitrogen as nitrates.

The test affords very useful confirmatory evidence of added water, and, in common with the freezing-point test, distinguishes actually added water from water normal to the milk, in contrast with the indirect evidence adduced or "presumed" from the figure for solids-not-fat, with or without comparison with the results given by an appeal sample. One is sometimes asked in Court if one can distinguish between actually added water and water normal to the milk, and it is satisfactory to be able to affirm that one can, and has done so.

We thought it desirable to determine, upon an experimental basis, whether or not cows drenched with nitrates secrete it in their milk. Accordingly, with the kind assistance of the County Agricultural Organiser, we arranged to have two cows put on the maximum dose of "nitre" for seven days and to test their milk day by day. The dose of "nitre" given was 1/3rd oz. twice daily. With this heavy dose of nitrates we anticipated that the appearance of, at least, some trace would be evident in the milk after several days, and were prepared to note how long it persisted, but no trace of nitrates was found in any of the fourteen samples from the two cows.

With regard to the precautions to be observed in order to eliminate fictitious results we would emphasise the importance of ensuring that the filter paper used is free from nitrates. In the early days of our experiments we were greatly puzzled by obtaining positive tests with the large majority of samples. Investigations showed that these fictitious results were due to nitrates contained in the filter paper.

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### ON THE TESTING OF DISINFECTANTS.

IN a recent communication (ANALYST, 1931, 93), Patterson and Frederick give a reasoned and detailed description and discussion on the most recent Admiralty method for the testing of disinfectants, and at the same time criticise the Rideal-Walker method for a supposed lack of accuracy. It is not our desire in any way to enter into a discussion on the relative advantages and disadvantages of either method of testing; but, since we have had frequent occasion to use these and other methods for the determination of relative germicidal powers, a few points in the paper of Patterson and Frederick appear to us to be worthy of some comment. In the first place they rightly emphasise the necessity of rigorous standardisation of materials and technique; the variable coefficients cited by these authors, as obtained by the Rideal-Walker method, were, in fact, from a series of tests which emphasised the necessity for such rigorous standardisation in the case of this method. For uniform samples of disinfectant which do not change or separate with age, it is, we think, fairly definitely established, that, with the modern Standardised Rideal-Walker technique, an error of  $\pm 5$  per cent. in the Rideal-Walker figure is the usual tolerance, whilst the frequent occurrence of  $\pm 10$  per cent. would indicate some departure in detail from the test. We should be surprised if any other test, including the Admiralty method, could be claimed, at the present time, to be so standardised as to bring the range of accuracy down to these limits.

Since the paper of Patterson and Frederick is published by permission of the Director of the Medical Department, Royal Navy, it is presumably an authoritative account of the correct details of the Admiralty Method, information on which, as printed on contract forms, has hitherto been somewhat meagre; and,

indeed, there are still some points on which further information would be desirable. Some authorities, believing that the disadvantages of comparison of disinfectants by a method which gives coefficients confined to a small numerical scale, are outweighed by advantages in attempting to reproduce the form of medium in which the actual operation of disinfection on a large scale is frequently carried out, add organic matter to the disinfectant dilutions. The Admiralty employs a gelatin, rice starch, salt mixture. It would be desirable to have more information on the colloid reactions occurring on the addition of the rice starch to the gelatin dispersion, as, presumably, the gelatin is partly adsorbed by the starch. In the light of Nugent's work (*Trans. Faraday Soc.*, 1922, 17, 703), the question of the time of contact may not prove immaterial, whilst the purity and effect of the rate of hydrolysis of the gelatin, and the  $pH$  of the artificial sea-water, are all variables which may have an important bearing on the possible limits of accuracy of the test. We would certainly agree that the gelatin, rice-starch suspension is superior to urine, faeces or pus, one of which is employed in the Martin-Chick test, and is, at the moment, adopted by another Government Department.

The authors, on pp. 98, 99, lay stress on the need of accurate dosage of culture to diluted disinfectant, and suggest that the dosage employed in the Admiralty Test, *viz.* 0.25 c.c., works satisfactorily. It may, however, be observed, on p. 102, that the actual dosage varies from 0.25 c.c. of culture to 4.5 c.c. of disinfectant, to 0.25 c.c. of culture to 5.4 c.c. of disinfectant dilution, a difference of nearly 2 per cent. This introduces a grave source of error, which should be eliminated in the final draft of the test.

There is also another point in the description of the test on which more clarity would be desirable so as to ensure more uniformity in the results obtained by different observers, namely, the material for making the broth. In the Rideal-Walker method, Lemco is specified. Whilst it is generally agreed that this material is not perfectly uniform in respect of the salt, creatine and creatinine, it is at least more uniform than a bullock's heart or a beef-steak. A meat medium produced on a large scale must always be more uniform than single ingredients bought casually from time to time. Among the users of the Admiralty tests great uncertainty still exists as to whether bullock's heart is employed or beef-steak, as used by McIntosh and Smart. One manufacturer actually possesses a letter from the Admiralty stating that bullock's heart is used, the variability of which has long been established.

Finally, two minor points might be noted. On p. 100, in a discussion on the time given for the test, the reader would gather that time intervals up to  $12\frac{1}{2}$  and 15 minutes are necessary in the Rideal-Walker method, and that some latitude is permissible in the choice of the time periods found necessary for killing, from which the coefficient is obtained. Such extended periods have not been used in the Rideal-Walker technique for many years, and it is incorrect to state that a choice of the coefficient is permissible, for a definite statement is made in respect of the technique of the Rideal-Walker method to guard against the possibility of such action by the tester, namely, that the concentration of disinfectant and carbolic acid which gives life in  $2\frac{1}{2}$  and 5 minutes and death in  $7\frac{1}{2}$  and 10 minutes shall be chosen.

It is to be hoped that the publication of further details of the Admiralty method will remove some of the uncertainties which, at present, confront those who wish to use it.

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## THE VALUATION OF CARBOLIC POWDER.

WITH reference to the note by the Borough Analyst for Stepney (ANALYST, 1931, 31), there are one or two points upon which I should like to comment. Among the unsatisfactory powders condemned was one stated to consist of spent gas-lime containing 9 per cent. of naphthalene. Such a powder would not, of necessity, be unsatisfactory bacteriologically, since it might have a Rideal-Walker coefficient at least twice that of a powder complying with the specification.

Some time ago we carried out a long series of investigations on the bactericidal value of various coal-tar products, and found that powders of similar composition to that quoted gave Rideal-Walker coefficients up to 4, whereas a powder containing 15 per cent. of cresylic acid gave average results of 0.75. The character of the tar-acids in these powders has, of course, a very great influence on the Rideal-Walker coefficient; the tar acids having a boiling point range from 200° to 300° C. showed a Rideal-Walker coefficient as high as 20.

In our Rideal-Walker coefficient tests we followed the technique laid down in the booklet "Approved Technique of the Rideal-Walker Test" (H. K. Lewis). This does not give any special directions for powders; in our tests a 1 per cent. suspension of the powder was prepared, and further dilutions were made from this, the 1 per cent. dilution being carefully shaken to ensure fair sampling of soluble and suspended material.

Analysts and others who are responsible for the control of disinfectants supplied to local authorities would be well advised to insist that a Rideal-Walker coefficient be included in the tender, and the chemical specification be so modified that a content of 15 per cent. "cresylic acid" be no longer regarded as the criterion of quality. Such an amended specification would prevent the condemnation of powders which, in actual use, are more efficient than those complying with the rather antiquated specification that "Carbolic Powder must contain not less than 15 per cent. of tar-acids calculated as cresylic acid."

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## Notes from the Reports of Public Analysts.

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### COUNTY OF ESSEX.

#### REPORT OF THE COUNTY ANALYSTS FOR THE FOURTH QUARTER, 1930.

**TIN IN CHEESE.**—Among the samples of cheese examined during the quarter were 35 samples of what is known as "packet" or "processed" cheese, about which a good deal of alarm was recently raised by some observations in a report issued by the Ministry of Health in regard to metallic contamination. These cheeses, of which there are very many varieties, prepared at home and abroad, are wrapped

in tin-foil which is a potential source of metallic contamination, though not necessarily to a serious extent. Much no doubt depends first upon the actual composition of the foil itself, and, secondly, upon the way in which it is prepared. Some packers, we understand, take care to have the foil treated by an invisible process which is akin to the process of lacquering, in order to inhibit as far as possible actual contact between the metal and the cheese. Cheese is a substance which necessarily contains free acid (lactic acid), which must have some tendency to attack metal. The extent to which this may take place will depend a good deal upon care in selection of the foil used, as already indicated, and in cases in which the nature or condition of the foil may invite attack, the extent to which this takes place will be dependent largely upon the age of the cheese, that is to say, the time which has elapsed between its being wrapped in the foil and delivered on to the plate of the consumer. We think that some of the more sensational cases that have been referred to must have related to cheese that had been packed for a long time before being examined.

Of the 35 samples submitted from the various county inspectors during the quarter, no sample was entirely free from tin. Expressing the quantities found in the terms in which tin contamination in preserved foods is recorded, namely, the number of grains or fraction of a grain per pound, we have found in the samples submitted to us a variation from 0.3 grain per pound as a minimum up to 5.5 grains per pound as a (quite exceptional) maximum.

It is difficult to draw a logical line between what might be regarded as passable and what might be regarded as excessive in contamination of this kind. As long ago as 1908 the Ministry of Health undertook an extensive investigation into the question of the contamination with tin in what are ordinarily described as "tinned" or "canned" goods, that is, preserved meat products, soups, fish, vegetables, fruit, etc., put up in tin, or rather tin-plated containers; and, as an outcome of that investigation, it was suggested that when there was an approach to 2 grains per pound in such articles as were under survey, further examination and consideration might be called for on the ground of potential deleteriousness to health. A good deal of discussion was contained in the Ministry's report on the question of the influence on health of small quantities of tin, but the evidence cited was not very definite, and, in some cases, conflicting.

Although no definite regulation has been made in limitation of such contamination, it has, since the report referred to, been generally accepted that any contamination up to 2 grains per pound need not be taken too seriously, while, at the same time, anything above this arbitrary figure of 2 grains per pound should be regarded as excessive. As this generally received limitation applies to articles which are usually consumed at any given meal in far larger quantities than cheese, it would seem that, in the case of cheese, the presence of tin, at any rate up to 2 grains per pound, need not be taken as being a grave contamination, but, having regard to the fact that, in this recent investigation of our own, 28 samples out of 35—or 80 per cent. of the whole—showed a contamination which did not exceed 2 grains per pound, anything above this figure may reasonably be looked upon as unnecessarily and undesirably excessive.

The 28 samples in which the contamination did not exceed two grains per pound included samples prepared from many varieties of cheese, *viz.* many cheeses of the Cheddar variety, both home-made and "Colonial," and a large proportion prepared from Gruyère cheese in Switzerland, France and elsewhere. The seven samples which contained more than two grains per pound, and which we have recorded as unsatisfactory, were all samples of Gruyère cheese (either Swiss or

French), the contamination, expressed as grains of tin per pound of cheese, being 2.2, 3.0, 3.0, 3.0, 4.0, 4.5, 5.5 grains, respectively.

It seems probable that the wide attention which has lately been directed to this question will stimulate any packers who may have been lax in the matter to pay increased attention to the preparation of the foil used, and also, as far as possible, to influence those concerned in the channels of distribution with a view to shortening, as far as possible, the interval between packing and the delivery of the cheese to its final customer.

BERNARD DYER.  
GEORGE TAYLOR.

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### THE ARTIFICIAL CREAM ACT, 1929. AN APPEAL CASE.

KEATING *v.* LYONS.

ON 6th February, an Appeal was heard in the King's Bench Division (before the Lord Chief Justice, Mr. Justice Avory, and Mr. Justice Macnaghten) from a conviction by the Swindon magistrates of a firm for selling Swiss rolls, chocolate cream sandwiches, and vanilla cream sandwiches containing a substance purporting to be cream, but which was alleged not to be cream as defined by the Act (ANALYST, 1930, 55, 689).

Mr. Roland Oliver, K.C., for the appellants, contended that the Act did not apply to a composite article, such as had been sold, but only to cream sold separately as cream. In his submission, the Act was not meant to apply to compound articles such as Swiss roll or chocolate cream, which had long been sold without complaint.

The Lord Chief Justice (Lord Hewart) gave the decision of the Court, which was that the case should be sent back to the Swindon justices for them to consider whether the articles in question were sold as substances purporting to be cream as defined in the Act.

### MILK: THE FREEZING-POINT TEST.

ON February 12th, the South Staffordshire Stipendiary (Mr. B. G. Grimley) delivered his reserved judgment in a case in which a dairy farmer was summoned for selling adulterated milk.

The solicitor for the defence had endeavoured to establish, through the evidence of employees, that no water had in fact been added; and, secondly, by expert evidence, that the legal presumption, based upon the analyst's certificate, was not justifiable, since the conventional tests at present employed were in themselves inconclusive.

The Stipendiary referred to the report of the Standing Committee on the Law relating to Milk (1930) (*J. Ministry Agric.*, 1930, p. 386), which said that the result of an analysis of a single sample was never proof of adulteration, and that the only proof



was the evidence of an eye-witness (which one never had), or by circumstantial evidence based on the analysis. It was his duty, he said, to take the law, not as the circular suggested it should be, but as it was at present. It was certainly significant that when no one watched the milking, the milk was presumably adulterated; whereas, when the milking was watched, the milk was all right. After an "appeal to the cows" on the day after the summons, the milk was found to be of good quality, and there must have been some unnatural cause for this. It has been laid down that, in order to succeed, the defence must satisfy the Court beyond doubt that no water had been added to the milk, and the evidence must be positive, clear and complete, leaving no gap from the moment at which the milk was taken from the cows to the moment at which it was handed to the purchaser; and where a doubt remained, it was quite clear that the burden of proof had not been discharged, and the defence had failed. In this case a wagoner about the farm had not been called as a witness, and the evidence of one brother was contrary to that of the other. He was bound by the decision of the Lord Chief Justice; the defence did not leave his mind free from doubt, and, therefore, failed.

Dealing with the scientific evidence, the Stipendiary said that it had complicated the case, and was outside his experience in such cases. "What I certainly do hope," he said, "is that, in subsequent cases, I shall have evidence about the freezing-point test; and, in the meantime, if Mr. Hanley (who gave evidence for the defence) meets with any herd of cows similar to the one he mentioned, which gave an exceptionally low freezing point, I hope he will communicate with Mr. Jones (the Staffordshire County Analyst), and that Mr. Jones will join him in an investigation." In view of the fact that the defendant knew nothing about the matter, he would deal with him under the First Offenders' Act, not recording a conviction, and reducing the costs to £25 4s.

## Department of Scientific and Industrial Research.

### THE INVESTIGATION OF ATMOSPHERIC POLLUTION.

#### REPORT ON OBSERVATIONS IN THE YEAR ENDING 31ST MARCH, 1930.

(SIXTEENTH REPORT.)\*

THE general arrangement of the Report follows that of 1929 (ANALYST, 1930, 55, 755). A map is included to show the positions of the contributing stations, and data have been collected as to steps taken by co-operating authorities to abate the smoke nuisance, and these are discussed.

#### Report of the Atmospheric Pollution Research Committee.

In addition to the usual investigations, the Atmospheric Pollution Committee have initiated an enquiry into the spread of pollution, and measurements are being made at Norwich, as a centre isolated enough to prevent interference from other sources of pollution. Daily observations on wind direction and velocity are taken, followed by pollution measurements at selected spots downwind, made by means

\* Published 27th February, 1931, pp. 74. Obtainable from H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 4s. net.

of an Owens Jet Dust Counter. The data obtained will be analysed when a year's cycle is completed. Suggestions as to the best method of protecting the deposit gauge bottles from being burst by frost are being tested.

**Superintendent of Observations.**—During the year there were set up 8 new deposit gauges, making a total of 84 maintained by 38 authorities, and 11 automatic filters maintained by 6 authorities. The maximum and minimum monthly deposits in metric tons per 100 sq. km. were:—*Tar*: Salford, Regent Road, 60; Glasgow, Bellahouston Park, 2; *Other insoluble carbonaceous matter*: Rochdale, St. Mary's Church, 386; Birmingham, West Heath, 39; *Ash of insoluble matter*: Rotherham, Town Hall, 819; Garston, Watford, 61; *Ash of soluble matter*: London, Kew, 481; Leicester, Western Park, 85; *Total solids*: Liverpool, Netherfield Road, 1840; Leicester, Western Park, 304. The total solids comprised:—*Sulphates*: Huddersfield, Cooper Bridge, 300; Leicester, Western Park, 36; *Chlorine*: Salford, Regent Road, 119, Edinburgh, Princes St. Gardens, 15; and *Ammonia*: Huddersfield, Cooper Bridge, 61; Bournville, 1; *Rainfall*: Rochdale, 101 mm.; London, South Kensington, 40 mm.

The different observation stations have been classified A—D on the basis adopted in previous reports; and in Table II tar, other carbonaceous matter, insoluble ash, loss on ignition and soluble ash are classified under the different letters. Tar shows a reduction in 21, an increase in 13 out of 38 stations; other carbonaceous matter, a reduction in 23, an increase in 16 of 39 stations; insoluble ash, a reduction in 20, an increase in 17; loss on ignition, a reduction in 28, an increase in 11; soluble ash, a reduction in 28, an increase in 7; and total solids, a reduction in 24, an increase in 14 stations all of 39 stations.

On the whole, the current year shows a general improvement in the purity of the air, but tar and sulphates, regarded specifically as combustion products, increased in 1 Edinburgh and 2 London stations.

**AUTOMATIC FILTER RESULTS.**—The fact that the maximum concentration of sooty impurity for Westminster Bridge station, although occurring at 9 a.m. last year, was this year maintained on to 10 a.m., is regarded as probably due to incidence of foggy weather. The graph of the 3 days of London fog of January 7th–9th, 1929, shows the maximum at 10 a.m. for the 7th and 8th, and 4 to 5 p.m. for the 9th, owing to the banking up of the smoke, due to insufficient ventilation, with consequent delay of the maximum. The maximum at Kew for "Z" days\* only was at 5 p.m., and at South Kensington the second p.m. maximum for ordinary days was higher than the 9 a.m. maximum, which was markedly different from what was observed in 1929.

**MEASUREMENTS OF DAYLIGHT.**—The measurements carried on at Leeds are set out as daily average figures for each month at 2 stations, and it was found that the measurements at the centre of the city were consistently lower than those about 2½ miles away.

**ULTRA-VIOLET RAYS.**—The measurements at Sheffield and Rotherham show that in the case of Surrey Street, Sheffield, no ultra-violet rays were present in November and December.

**Appendix.**—This discusses observations on ultra-violet light in Rochdale and other places. There is evidence that the great loss of ultra-violet light in the middle of the week is mainly due to factory smoke, for non-factory towns have most light in the middle of the week and least on Sundays. The power of the

\* "Z" days are any which show, at any time of the day or night, a concentration of 4 or over on the scale of shades, or 128 mgrms. per 100 cb. metres.

ultra-violet rays, measured by their fading action per hour, is not uniform, but depends on the season in such a way that it is proportional to the sine of the altitude of the sun.

**DEPOSIT OF MICRO-ORGANISMS AT SOUTHPORT.**—In March, 1930, a deposit fell over everything in the neighbourhood of Southport, and for an area of about 10 square miles the deposit is described as "remarkably thick." The dust, examined immediately after falling, was found to consist of algae, which are common epiphytes on tropical leaves, but are only found in this country on the windward side of rocks in mountain districts, and they have been noticed in the Peak district. At the time of the deposit the wind was from that direction, and it is possible that the algae, which are propagated by yellowish brown spores, which under suitable conditions grow as filaments, encountered soot particles which adhered to them, as the microscopical appearance of the dried deposit showed adhering bodies.

The usual detailed General Deposit tables are included in the Report.

D. G. H.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Physico-Chemical Constitution of Spray-dried Milk Powder. Fat in Spray-dried Milk Powder.** L. H. Lampitt and J. H. Bushill. (*J. Soc. Chem. Ind.*, 1931, 50, 45-54T.)—The "free" fat of a milk powder is taken to be the fat extractable under the authors' standard conditions by organic solvents, and on spray-process milk, calculated on total fat, it varied between 3.33 and 14.22 per cent., and on roller-process powder, between 91.6 and 95.8 per cent. To 2 grms. of powder in a glass-stoppered 250 c.c. bottle are added 100 c.c. of carbon disulphide or other solvent, and, after standing for 18 hours with gentle shaking during the first hour, the contents of the bottle are filtered through a Green's fluted filter paper (591½, 19.5 cm. diameter) into a 50 c.c. graduated flask, the first few c.c. being disregarded and the level adjusted by a capillary tube. The contents and washings from the flask are then poured into a weighed Soxhlet flask, dried and weighed. The residue is the "free" fat from 1 gm. of powder, and in the case of substances all the fat of which is normally extractable, the results agreed well with those obtained for the same substance by the Soxhlet process. If the surface of the spray-process particles is increased by grinding (3 hours), the "free" fat may be increased up to 83 per cent. of the total fat, but the fat is practically all "free" in the roller-process powder. The reasons for these differences are discussed. When milk powder absorbs moisture it first becomes clammy, and at the next stage is distinctly dry, hard and powdery to the touch, and this corresponds with the "freeing" of the fat. The definite range of moisture content (about 8.6 to 9.2 per cent.) at which the fat is freed is called the "critical moisture" content,

and is accompanied by the crystallisation of the amorphous lactose in the spray-process milk, but, apparently, a freshly-made powder reacts more slowly than an older one. On drying out such absorbed moisture the powder does not revert to its original state; and the critical moisture range, while independent of the fat content as such, was found to be proportional to the amount of solids-not-fat in the powder. While no theory is put forward as to the structure of the milk powder particle, it is clear that the lactose, in its amorphous state, has a marked effect on the availability of the fat to solvents, and that crystallisation of the lactose appears to result in freeing the fat.

D. G. H.

**Determination of the Ash of Bread.** F. Bernardini and E. A. Gauthier. (*Giorn. Chim. Ind. Appl.*, 1931, 13, 7-9.)—In the ordinary method used for determining the ash of bread the preliminary digestion with water does not extract all the sodium chloride, and the subsequent incineration of the residue for 15 minutes at a dull red heat results in a black ash, and, if the heating is prolonged, loss of chlorine occurs. Satisfactory results are obtained by Cutolo's method (*Boll. Soc. Naturalisti Napoli*, 1914, 26, 69), in which the ash is determined on the bread alone, and also on a second sample after treatment with alcoholic potassium hydroxide; the chlorine in each residue is determined by Volhard's method, and the difference between the two, calculated as sodium chloride, added to the weight of the first ash. This procedure is, however, laborious if many samples have to be tested, and equally good results may be obtained as follows:—Five grms. of the finely powdered dry bread, weighed in a platinum dish, are transferred to a beaker, treated with 3.2 c.c. of water, and, after about 10 minutes, when the bread has swollen, with 50 c.c. of 99.5 per cent. methyl alcohol. The mass is stirred occasionally during an hour, after which the liquid is decanted on to a filter, and the residue twice treated similarly with one-half of the above quantities of water and methyl alcohol. The residue in the beaker is dried in an oven and then restored, with the filter, to the platinum dish, in which it is charred over a naked flame, incinerated in a muffle furnace at a low red heat, and weighed. This procedure gives the total ash, less sodium chloride, and thus furnishes information of use in judging the character of the original flour.

T. H. P.

**Essential Oils from Grain Germs: Improver for Bread and Food-stuffs.** (*Perf. Essent. Oil Record*, 1931, 22, 38.)—According to the process here described, which is the subject of a British patent application by M. Baumann, wheat germs contain, in addition to the oily and fatty constituents which rapidly produce rancidity in flour, etheric oils capable of imparting a pleasant flavour to bread. Such ethereal oils, which may be obtained, not only from wheat, but also from the germs of rye, barley, oats, maize, buckwheat, etc., and from such fruits as nuts, almonds, and coconuts, may be separated either by extraction and distillation processes, or exhaustion or solution processes with the aid of various organic solvents, or "enfleurage" processes. The oils may be incorporated directly in the flour, or may be mixed with the yeast or other products used in the manufacture of bread.

T. H. P.

**Ursolic Acid.** C. E. Sando. (*J. Biol. Chem.*, 1931, 90, 477-495.)—Sando (*J. Biol. Chem.*, 1923, 56, 457; ANALYST, 1923, 48, 496) reported the results of an investigation of the constituents of the wax-like coating on the surface of the apple, from which there was isolated, among other substances, a compound insoluble in petroleum spirit to which the name malol was given. It possessed the same formula,  $C_{30}H_{48}O_3$ , and characteristics as urson, originally isolated by Trommsdorff (*Arch. Pharm.*, 1854, 80, 273), from bearberry leaves, *Arctostaphylos uva ursi*, and exhibited the same general chemical properties as prunol, isolated from the leaves of the wild black cherry, *Prunus serotina*, by Power and Moore (*J. Chem. Soc.*, 1910, 97, 1099). In 1924, van der Haar (*Rec. trav. chim. Pays-Bas*, 1924, 43, 367) reported a re-investigation of urson, suggested the name ursolic acid as being in accord with its chemical character, and claimed for the substance the formula  $C_{31}H_{50}O_3$ . On the basis of this work he concluded (*Rec. trav. chim. Pays-Bas.*, 1924, 43, 542; 548) that malol, prunol and ursolic acid were identical. The formation of the acetylation products was also brought into question. Because of these discrepancies a more exhaustive chemical study of the three substances has been made, the results of which are given, and may be summarised as follows: Malol, prunol and urson are identical; van der Haar's conclusions in this respect are thus confirmed. The name, ursolic acid, has been adopted for the substance. The formula for ursolic acid appears to be  $C_{30}H_{48}O_3$ , and not  $C_{31}H_{50}O_3$ , as claimed by van der Haar. This conclusion is based on the results of 92 combustions, including those of the parent substance and many of its derivatives. The following compounds have been prepared and analysed: ursolic acid from three sources, the diacetyl derivative of ursolic acid, monoacetyl-ursolic acid, regenerated ursolic acid, methylursolate, monoacetylmethyl ursolate, regenerated methyl ursolate, phthalylursolic acid, phthalylmethyl ursolate and phenacyl ursolate. The average values of numerous combustions of these substances agree more closely with the  $C_{30}$  formula than with the  $C_{31}$  formula. The preparation of monoacetylmethyl ursolate, by treating monoacetylursolic acid with thionyl chloride and boiling the product with methyl alcohol, constitutes further confirmatory evidence of the hydroxy-acid structure of ursolic acid.

P. H. P.

**Origin, Occurrence and Detection of 2,3-Butyleneglycol in Wine and Fruit Wine.** J. Pritzker and R. Jungkunz. (*Z. Unters. Lebensm.*, 1930, 60, 484-488.)—The sample (25 c.c.) is evaporated in a dish on the water-bath to 5 c.c., which is transferred to a 50 c.c. Erlenmeyer flask, and 4 grms. of crystalline sodium carbonate added and dissolved by warming. The mixture is then cooled in ice, 0.5 c.c. of bromine water added, and, after 30 minutes at room-temperature, during which time it is shaken to remove carbon dioxide, the mixture is neutralised (to litmus) with 10 per cent. hydrochloric acid. Any remaining excess of bromine should be removed by a drop of concentrated sodium thiosulphate solution, and the potassium iodide and starch test applied. The diacetyl, produced by oxidation of the 2,3-butyleneglycol, is then detected by distillation with 50 c.c. of 30 per cent. ferric chloride solution as oxidising agent in a Reichert-Meissl-Polenske apparatus,

the distillate (20 c.c.) being collected in a mixture of 2 c.c. of 20 per cent. hydroxylamine hydrochloride, 3 c.c. of 20 per cent. sodium acetate and 1 c.c. of 10 per cent. nickel chloride solutions. Dimethylglyoxime is produced, and when the distillate separates into 2 layers, the characteristic crystals of nickel dimethylglyoxime are formed as a ring at the junction. The distillate may then be warmed in a water-bath at 80° C. for an hour, cooled, and the precipitate separated by filtration, washed with hot water and dried in the water oven till constant in weight. The factor 0.624 gives the weight of 2,3-buteneglycol. This procedure gives higher results than other methods, a value of about 0.6 per cent. being found for wine and fruit wine (Spanish Montagner), though theoretical considerations indicate the possible presence of 0.2 per cent. None was found in bottom-fermentation beer, but it is uncertain whether this is due to the difference in the nature of the sugars or of the yeast, or to the working temperature involved. The method serves as a specific distinction between fermented and unfermented wines, *e.g.* between true sweet wine (such as Malaga), from which alcohol has been removed, and wine prepared from unfermented grape or fruit must. The German official method for the determination of glycerin (*id.*, 1921, 13, 93) requires no correction for the presence of 2,3-buteneglycol. J. G.

**Oleic Acid Rancidity of Fats. II. Measurement of Rancidity.** K. Taüfel and J. Müller. (*Z. Unters. Lebensm.*, 1930, 60, 473-484.)—The literature dealing with qualitative tests for rancidity is discussed fully and critically, with particular reference to the effects of light and of interfering substances, and to the possible modification of such methods in order to obtain approximately quantitative results which will enable the evolution of rancidity to be followed. The Kreis test was found to give reproducible results if measurements were made by comparison of the resulting colour with the colours of a series of 12 photographic filters, ranging from the colour obtained from freshly-prepared ethyl oleate to the maximum colour obtainable from the strongly rancid ester. The colour match was made 2 minutes after mixing the reagents. The rate of production of rancidity, according to this method, attains a constant maximum (colour No. 12) after 14 days in the light, but increases very slowly and linearly in the dark, reaching only colour No. 1 after 25 days. The acid value increases almost linearly between the 8th and 25th day at a rate intermediate between those shown by the above Kreis tests. No relation between the 3 curves was detectable. Comparison was then made with the rate of absorption of oxygen (Genthe). The volumetric method, in which the change in pressure of oxygen in contact with oil was measured manometrically, gave unreliable results, and the increase in weight of about 2 grms. of oil (oleic acid and its ethyl ester) exposed to the air in a glass dish was preferred. Both compounds showed approximately the same rates of increase in weight, these being approximately constant (70.0 and 76.4 mgrms., respectively), after 70 (and up to 90) days. Although the method allows a wide range of time to be studied, unreliable results are obtained over the first 2 days. Comparison with the Kreis test for 1 to 20 days showed a much

more rapid increase in rancidity in this test, though both curves tend to approach the same value at the end of this period. It is probable that the different methods correspond with changes of different character. J. G.

**Oil of *Wrightia annamensis*, Dubard and Eberhardt; an Oil resembling Castor Oil.** M. L. Margailan. (*Compt. rend.*, 1931, 192, 373-374.)—The seeds of *Wrightia annamensis*, gathered in Tonkin as "cay thu muc" N.O. *Apocyanaceae*, had the following composition:—Moisture, 5.8; fat (to petroleum spirit), 36.1; protein, 29.1; cellulose, 5.1; ash, 4.3 and non-nitrogenous residue, 19.6 per cent. The ash contained alumina, 50.6; silica, 19.1; potash, 10.7; and phosphoric acid, 11.2 per cent. The oil is of a dark pomegranate-red colour; it is soluble in alcohol in all proportions and has,  $d_{20}^{20}$  C. 0.966, viscosity coefficient C.G.S. at 20° C., 6.76,  $n_D^{20}$  C., 1.480; flash point (open-dish method), 279° C.; ignition point, 320° C.; calorific value (bomb), 8720 grms.-cal.; saponification value, 184; iodine value (Hübl), 85; acetyl value, 127; unsaponifiable matter, 1.0 per cent.; free fatty acids (as oleic acid), 2.7 per cent. The oil showed no trace of drying in one month. The viscosity curve for varying temperatures was analogous to that of castor oil; the acetyl values of the two oils are similar; their combinations with sulphur, their polymerisation powers, and their soaps are closely alike, but *Wrightia* oil is more soluble than castor oil in petroleum spirit. A hydroxy-oleic acid, probably identical with ricinoleic acid, is the principal constituent of the fatty acids of *Wrightia* oil, and the presence of a small proportion of a hydroxy-linoleic acid is considered probable from the results obtained by fractional crystallisation of the barium salts and by fractionating the cooled fatty acids.

D. G. H.

**Nicotine Contents of Dutch Cigars.** A. Van Druten. (*Z. Unters. Lebensm.*, 1930, 60, 501-518.)—The methods and results of previous workers are discussed, and the highest, lowest and mean values of the nicotine contents found by them for ordinary, and partly and "wholly" denicotinised cigarette and cigar tobaccos are tabulated. Examinations of Bodnár, Straub and Nagy's micro-titrimetric method (*Biochem. Z.*, 1928, 195, 103), of Rasmussen's silicotungstic acid method (*ANALYST*, 1916, 41, 208) and of Pfyl and Schmitt's method (*id.*, 1927, 52, 728), showed that the second is usually reliable under the conditions specified (*loc. cit.*), but requires 10 grms. of tobacco, whilst the first gives high results, and the third, modified as follows, is to be preferred. The sample is ground in a mortar, 3.5 grms. mixed in a 200 c.c. distillation flask with 35 c.c. of water, and 25 grms. of sodium chloride and an aqueous paste containing 1 gram. of magnesium oxide added. The mixture is steam-distilled, 150 c.c. of distillate collected and neutralised to methyl red with 0.1 N hydrochloric acid, and the indicator removed by a drop of bromine water. Not less than 75 c.c. of 0.05 M picric acid solution are then added, the mixture cooled in water for 2 hours, and the precipitated nicotine dipicrate filtered on a platinum cone-filter (5.5 cm. in diameter) and washed with 4 c.c. of 10-fold diluted reagent, and twice with water. The filter and contents are then transferred to a 100 c.c. flask, 10 c.c. of water and 4 drops

of phenolphthalein (1:100) added, and the mixture titrated, with shaking, with 0.1 *N* sodium hydroxide solution, 25 c.c. of toluene being added when the red colour first appears just before the end-point. The factor 0.243 gives the percentage of nicotine. The results for 104 "normal" Dutch cigars varied from 0.5 to 2.7 (mean 1.42) per cent. on the dry tobacco, and were independent of size and price, whilst 0.57 to 1.53 per cent. was found in six cigars sold as containing "minimum" and "absolutely harmless" amounts of nicotine. The opinion is expressed that "nicotine-free" cigars should contain only traces of nicotine, and that 0.5 per cent. is a suitable upper limit for "denicotinised" cigars. J. G.

#### Importance of the Acidity of Tobacco for its Hygienic Evaluation.

**A. Fajtelowitz.** (*Z. Unters. Lebensm.*, 1930, 60, 518-523.)—The acidity of cigarette tobacco (*A*) is determined by mixing 10 grms. and 100 c.c. of water for 2 hours and titrating 100 c.c. of the strained liquor with 0.1 *N* sodium hydroxide solution. The basicity of the un-neutralised smoke (*B*) is given by the volume of 0.1 *N* sulphuric acid neutralised when the smoke from 10 cigarettes is drawn through the acid. The ratio *A/B* is an indication of the degree of mildness of the tobacco. By "smoking" cigarettes in a glass tube by means of an aspirated current of air, and analysis of the smoke before and after neutralisation, the following data were obtained for 6 tobaccos:—Nicotine in tobacco, 0.4 to 3.03 per cent.; nicotine in neutralised smoke (100 cigarettes), 0.2 to 1.66 per cent.; *A* 22 to 27.5; *B* 50 to 83; free bases in neutralised smoke (100 cigarettes), 0 to 30 c.c. of 0.1 *N* sulphuric acid. Similar values are also given for the tobacco after making alkaline, the nicotine and free bases in the neutralised smoke then being 0.3 per cent. and 51 to 85 c.c. higher, respectively. Attempts to correlate these data with the tastes of the corresponding tobaccos indicate that amino-bases are liberated during the smoking process and irritate the throat, but that their effect depends on the degree of acidity of the tobacco, *i.e.*, on the extent to which they are neutralised as the smoke traverses the cigarette to the mouth. Cigar tobacco has a lower acidity than cigarette tobacco, and is sometimes alkaline. J. G.

**Analysis of Hops.** **W. Wöllmer.** (*Allgem. Brauer- und Hopfen-Z.*, 1930, 70, 1531-33; *J. Inst. Brew.*, 1931, 37, 81-83.)—The following scheme, proposed by the author, has been adopted by the Analysis Commission of the German Brewing Institutes: **Moisture.**—Three grms. of finely ground sample are dried at 104° to 105° C. for 3 hours, as in the German method of malt analysis. **Extraction for Resins.**—Ten grms. of the sample are shaken for 2 hours in a bottle (with a stopper, if of rubber, of diameter less than  $\frac{3}{8}$  inch) with 100 c.c. of ether, and, after a period of rest of 30 to 60 minutes, according to the age of the hops, 50 c.c. of the top liquor are pipetted into a 200 c.c. conical flask and evaporated at 70° C. The last traces of ether are removed in a current of air free from carbon dioxide, contact of air with the hot resins being avoided as far as possible. The cold residue is then re-extracted with 20 c.c. of methyl alcohol, the mixture filtered through a paper (3 inches in diameter), and the filtrate and methyl alcohol washings



made up to 50 c.c. **Total Resins.**—Ten c.c. of this extract are pipetted into a weighed flask, the alcohol removed on the water bath, and the residue dried by passage of a current of dry carbon dioxide, rapidly at first, and then at 2 bubbles per second under a 20-inch vacuum (to remove hop-oil). This is followed by a current of air, and the flask is weighed, when the weight of extract (grms.)  $\times 102$  gives the percentage of total resins in the air-dry hops. **Soft (Hexane-Soluble) Resins.**—Fifteen c.c. of methyl alcohol extract are shaken by hand with 50 c.c. of hexane and 25 c.c. of water in a 150 c.c. stoppered cylinder for 5 minutes, and after separation, 40 c.c. of the upper (hexane) layer are pipetted into a weighed flask, and the same procedure followed as for the total resins (factor 85). **Humulone.**—If 1.07 grms. of pure crystalline lead acetate are rinsed into a 100 c.c. flask with methyl alcohol, followed immediately by 0.05 gm. of acetic acid, then 1 c.c. of the solution obtained after dilution to 100 c.c. with methyl alcohol will precipitate 1 per cent. of humulone in the sample under the following conditions:—The resin extract (10 c.c.) is warmed quickly at 50 to 60° C. in a 50 c.c. conical flask, and the above solution added till a slight excess of lead is present. This is shown by a brown stain at the junction on a piece of filter-paper of adjacent drops of supernatant liquid and 5 per cent. sodium sulphide solution, and, in the case of new hops, 4 to 5 c.c. may be added at once, followed by additions of 0.5 c.c. The precipitate (which dissolves in a large excess of reagent) is collected after 10 minutes on a Schott glass filter-crucible (No. 1 G4, emptying, if filled with water, in 20 to 25 seconds at a pressure of 1 atmos.), washed with 20 c.c. of methyl alcohol and once with ether, and weighed, after 30 minutes, at 105° C., followed by cooling in a desiccator (factor 65.2). The determination of the end-point of the precipitation, which depends on the age of the hops, is facilitated by centrifuging or by allowing the liquid to stand overnight; but in all cases the filtrate should be tested with 0.5 c.c. of reagent. **Hard Resins.**—The soft resins are subtracted from the total resins. The limit of error is about 0.3 per cent. in each case. Extraction of hops in a Soxhlet apparatus gives high values for the total resins. J. G.

**Determination of Arsenic and Mercury in Donovan's Solution.** T. T. Cocking. (*Quart. J. Pharm.*, 1930, 3, 575–577.)—**Arsenic.**—The determination of arsenic in Donovan's Solution should involve the determination of the total arsenic in addition to the determination of the arsenious iodide, because some of the latter becomes oxidised on keeping the solution. The following method is suggested: To 50 c.c. of Donovan's Solution is added 1 gm. of sodium bicarbonate; the solution is titrated with *N*/10 iodine and the result calculated to  $\text{AsI}_3$ . Starch is not used to indicate the end-point, as this is quite sharp to one drop of the volumetric solution. Forty-five c.c. of hydrochloric acid (31 to 32 per cent. HCl) are now added, and the mixture is kept at the laboratory temperature for 10 minutes and then titrated with *N*/10 thiosulphate, without using starch. The total arsenic thus indicated is calculated to the equivalent of  $\text{AsI}_3$ . The solution *must not* be diluted with water before the titration. It is pointed out that deviation from this method for the determination of total arsenic may lead to the formation

of yellow arsenic iodide which would obscure the end-point. The author notes that the formation of a brilliant yellow precipitate of arsenious iodide, when an excess of conc. hydrochloric acid and potassium iodide solution are added to a solution of arsenious acid, is a sensitive reaction for arsenic.\*

**Mercury.**—Fifty c.c. of Donovan's Solution are rendered strongly ammoniacal and the mercury precipitated by hydrogen sulphide; the mercuric sulphide is filtered off on a Gooch crucible, washed with ammonia, dried at 120° C., and the weight calculated to the equivalent of mercuric iodide. The method of the United States Pharmacopoeia is stated to give low and variable results. S. G. C.

\**Abstractor's note*:—Papers by G. Bressanin on the qualitative and quantitative aspects of this arsenious iodide reaction appeared in *Boll. Chim. Farm.*, 1911, 50, 691, 727; and *Gazz. Chim. Ital.*, 1912, 42, 456.

## Biochemical.

**Phosphorus Distribution, Sugar and Haemoglobin in the Blood of Fish, Eels and Turtles.** C. M. McCay. (*J. Biol. Chem.*, 1931, 90, 497–505.)—Little information is available concerning the chemical composition of the blood of the lower vertebrates, owing, probably, to the general difficulty of obtaining unclotted samples. The blood of all species that have nucleated erythrocytes has the common property of very rapid clotting after it is removed from the body. Among these, the blood of the turtle is probably the slowest to clot, whilst fish and eel blood are at the other extreme. A study has now been made to determine the phosphorus distribution in the blood of some of the lower cold-blooded vertebrates. A few other data, such as those upon the phosphorus distribution in the blood of the cow, have been incorporated in order to show the contrast in composition of two very different vertebrates. Values upon the sugar and haemoglobin of blood of these lower vertebrates have also been included in the cases in which they may throw some light upon the variations in phosphorus that have been discovered. The results show that the total phosphorus in the blood of animals with nucleated erythrocytes is higher than that in blood with non-nucleated cells. The blood of carp and pike has approximately four times as much phosphorus per unit volume as beef blood. The plasma phosphorus of pike blood is higher than that of carp; the blood sugar is also higher in pike blood. This may be a species characteristic related to activity, or may be associated with the differences of the two species in their resistance to asphyxiation. The phosphorus distribution in turtle blood differs from that of fish blood in the low values for the plasma constituents. The phospholipids of turtle blood are especially low. These are associated with the low cholesterol values that have been shown previously in turtle blood. The blood of eels is lower in phosphorus than that of fish blood, but higher than that of cattle. The blood changes that accompany the death of the lamprey-eels after spawning show some lowering of the phosphorus values, but the changes are chiefly concerned with glucose and haemoglobin decreases. Blood with large nucleated cells contains the same amount of total phosphorus as that with small cells. The plasma of both fish and turtle blood is slightly yellow, like that from the cow.

P. H. P.

**Colorimetric Determination of the Tyrosine and Tryptophan Content of Various Crude Protein Concentrates.** W. D. Mcfarlane and H. L. Fulmer. (*Biochem. J.*, 1930, 24, 1601-1610.)—In the course of investigations on poultry nutrition it was desired to know the tyrosine and tryptophan content of various crude protein concentrates. Though the colorimetric methods have sometimes given low values, and are open to the usual objections to colorimetric methods in general, they were the only methods which could be satisfactorily applied to the determination of tyrosine and tryptophan in a routine way, and it is doubtful whether any of them are absolutely reliable for determinations on purified proteins. The colorimetric method of Folin and Ciocalten (*J. Biol. Chem.*, 1927, 73, 627) and other methods were tried. The tyrosine and tryptophan content of the proteins of buttermilk powder has been found to be much higher than that of the other crude protein materials investigated. No very appreciable difference in the tyrosine content of fish meal, cod-liver meal, meat meal and tankage was found. Conflicting results as to the tryptophan content of the proteins of fish meal and meat meal, depending upon the method of determination, have been found. In general, the tryptophan content of fish meal was found to be higher than that of meat meal. The tryptophan content of tankage has invariably been found to be much lower than that of fish meal and meat meal. The limitations in the methods described for the determination of tryptophan leave some doubt as to the actual tryptophan content of any of these materials, particularly in the case of the cod-liver meal proteins. The alkali digest of these crude protein materials contains some substance, or substances, precipitable by mercuric sulphate and giving a blue colour with the phenol reagent, which, unlike tryptophan, is soluble in toluene. This unknown chromogenic substance does not appear to be indole, as Kraus (*J. Biol. Chem.*, 1925, 63, 157; *ANALYST*, 1925, 50, 246) has concluded. The results obtained after extraction of the alkali hydrolysates with toluene would appear to represent the true tyrosine and tryptophan content of these protein concentrates.  
P. H. P.

**Presence of Allantoinase in Fungi. [Test for Glyoxylic Acid.]**  
A. Brunel. (*Compt. rend.*, 1931, 192, 442-444.)—To detect allantoinase in fungi, a weighed quantity of the material is treated with an equal weight of anhydrous glycerin for 12 hrs. in an ice-chest, then with an equal weight of water for 6 hrs., and centrifuged. To 100 c.c. of 0.2 per cent. allantoin solution are added 25 c.c. of the centrifuged liquid, 0.125 grm. of ammonium bicarbonate and 1 c.c. of chloroform; a control, prepared similarly, but after heating the centrifuged liquid at 100° C. for 30 mins., is treated in the same way. Both preparations are kept at 40° C. for 12 hrs., and are then rendered distinctly acid with 5*N* hydrochloric acid and heated at 100° C. for one minute. To detect the glyoxylic acid formed, together with urea, by hydrolysis of the allantoic acid, 2 c.c. of each of the two liquids are treated with 4 drops of 1 per cent. phenylhydroxylamine hydrochloride and, after cooling, with 2 drops of 5 per cent. potassium ferricyanide solution and 3 c.c. of concentrated hydrochloric acid; an intense red coloration indicates the presence of

glyoxylic acid, the control remaining colourless. To detect the urea, 3 c.c. of each of the two liquids are neutralised with potassium hydroxide and treated with double the volume of acetic acid and with 5 per cent. (of the total volume) of a 10 per cent. solution of xanthydrol in methyl alcohol; the characteristic precipitate of the xanthydrol derivative indicates the presence of urea, the control remaining clear. In addition, the silver salt of allantoic acid may be prepared from the allantoin solution after fermentation for 48 hours at 40° C. This is defecated with 1 grm. of solid silver nitrate and filtered, the cold filtrate being treated with solid mercuric acetate and the precipitate formed separated by centrifuging, washed, and decomposed by hydrogen sulphide in presence of water (15 c.c.). After expulsion of the excess of hydrogen sulphide by means of a stream of air, the liquid is filtered, neutralised, and treated with silver nitrate. The silver allantoate precipitated, when crystallised from boiling water, forms groups of microscopic needles; it contains 38.12 per cent. of silver. By the above means the presence of allantoinase in 67 different fungi has been ascertained.

T. H. P.

**Reaction of Antimony Trichloride with Cod-Liver Oil and its Unsaponifiable Fraction.** E. L. Smith and V. Hazley. (*Biochem. J.*, 1930, **24**, 1942-1951.)—The authors have found that the unsaponifiable fraction of cod-liver oil gives with antimony trichloride in chloroform a blue colour proportional to its concentration. The line representing the dilution effect for the total unsaponifiable fraction is tangential at the origin to the dilution curve for the corresponding cod-liver oil. The chromogen is more stable than has sometimes been supposed, and there is no particular difficulty in obtaining practically complete extraction of the total unsaponifiable matter from cod-liver oil. The unsaponifiable fraction can be extracted almost without loss with ether, ethyl acetate, chloroform or petroleum spirit under suitable conditions. Norris and Church (*J. Biol. Chem.*, 1930, **85**, 477; **87**, 139; *ANALYST*, 1930, **55**, 204, 458) did not extract the whole of the chromogen from their saponified oil. A method is described in detail for carrying out the colour test on the unsaponifiable fraction extracted with chloroform. The colour developed is somewhat difficult to match against Lovibond glasses. Instead of the purplish blue given by cod-liver oil, the colour is almost invariably greenish blue, and is matched by a combination of blue and yellow glasses in the ratio of 10 to 4, very approximately.

P. H. P.

**Anti-scorbutic Fraction of Lemon Juice.** IX. S. S. Zilva. (*Biochem. J.*, 1930, **24**, 1687-1698.)—This paper deals in the main with two aspects of the antiscorbutic factor, namely, its fractionation and the mechanism which controls its spontaneous inactivation. Several years' work on the fractionation of the antiscorbutic factor is reviewed, and it is shown that variable activity is obtained under, presumably, the same conditions of fractionation. It is necessary to exercise caution in order to interpret correctly the available evidence on the subject. Batches of lemon juice sometimes yield fractions of low or no antiscorbutic activity when equal volumes of a saturated neutral lead acetate solution are used in the manipulation. It is possible, however, by adjustment of the quantity of the

precipitating agent to obtain from the same juice preparations of high activity. Therefore, in each case the conditions must be adjusted in order to obtain uniform high activity. Fractions, of which the phenolindophenol decolorising capacity falls below a certain limit, are found antiscorbutically inactive. This reducing property is not an index of antiscorbutic activity itself, but serves rather as an indication that the vitamin has been protected from inactivation. The reprecipitation of an antiscorbutic fraction from lemon juice or from cabbage juice with lead acetate in the neutral or slightly alkaline zone ( $pH$  8–9) yields an inactive preparation. The addition of an ethereal extract from autoclaved lemon juice, which decolorises iodine, but not phenolindophenol, of quinhydrone or of benzoquinone to unheated lemon juice accelerates markedly the destruction of the reducing principle and of the antiscorbutic factor in a neutral medium in the presence of air. Possibly a substance of phenolic character, oxidisable in the air, is formed in the process of autoclaving. This compound in its oxidised form destroys, in conjunction with the peroxidase present in the juice, the reducing principle that acts as an agent for the protection of the antiscorbutic factor. Decitrated lemon juice kept aerobically at  $pH$  0.6–0.8 for 7 days does not lose its reducing capacity for phenolindophenol to a greater extent than when kept at  $pH$  7, and shows little loss of its antiscorbutic activity. A discussion of the results obtained is given.

P. H. P.

**Toxicity of Vitamin D.** J. B. Duguid. (*Lancet*, 1930, 219, 983–985.)—

Although there is a wide margin of safety between the therapeutic dose of vitamin *D* and the dose which may prove toxic, the drug is remarkably potent, so that both care and knowledge must be exercised in deciding suitable dosage. The amount of ergosterol used gives no indication of the amount of vitamin *D* included, uniformity of production being obtainable only by a careful control of technique such as has not been observed in the past. As regards rats, the most useful information is furnished by experiments in which the substance is administered in quantities representing multiples of the antirachitic or curative dose—the rat unit of vitamin *D*. This dose is determined biologically by feeding young standard rats on a standard rachitogenetic diet for 3–4 weeks and then adding the vitamin in measured doses so as to ascertain the minimum daily dose which will effect healing in 10 days. Healing is estimated by calcification of the bones, which may be detected by radiological examination or by the “line test.” Deficiency in other vitamins appears to contribute in some measure to the toxic effects of vitamin *D*, but as yet the evidence on this point is not quite clear.

The Council of Pharmacy and Chemistry of the American Medical Association have adopted the name “viosterol” for a preparation of irradiated ergosterol standardised in its antirachitic properties by comparison with a certain standard, potent cod-liver oil, this preparation (in oil 100 D) containing about 1333 curative rat units per gm. Hess and his co-workers find that 10 drops daily of this viosterol represent the minimum prophylactic dose for infants, and that healing invariably results when from 30 to 60 drops daily are given.

Until recently there has been, in this country, no officially recognised standardisation of the various preparations of irradiated ergosterol obtainable, and the adjustment of the strength of the preparations has been at the discretion of the manufacturers, on whose directions the physician has mainly depended. The British Pharmaceutical Society has now, however, suggested the use of the Coward unit, based on a certain preparation of irradiated ergosterol, 0.0001 mgrm. of which, in daily doses, brings about healing in standard rachitic rats in 10 days. The general adoption of this rat unit is highly desirable, and there is a pressing need for the official standardisation of all preparations on the market and for an authoritative pronouncement on the proper dosage for therapeutic purposes.

T. H. P.

## Bacteriological.

**Selective Fermentation. II. Fermentation of Sugar Mixtures by Sauterne Yeast.** H. Sobotka and M. Reiner. (*Biochem. J.*, 1930, **24**, 1783-1786.)—Sobotka and Reiner (*Biochem. J.*, 1930, **24**, 926; *ANALYST*, 1930, **55**, 712) reported that two strains of "Sauterne" yeast preferred glucose to fructose in the fermentation of a glucose-fructose mixture. The value of  $K_{G/F}$  was similar to that of all other strains of *Saccharomyces* investigated. This was at variance with the findings of several French workers. The authors have now been able to secure a pure culture of authentic Sauterne yeast, and have repeated the work. The results show that genuine Sauterne yeast ferments fructose preferably to glucose in a mixture.  $K_{G/F}$  varies from 1.2 to values above 10 according to adaptation phenomena. This yeast ferments fructose alone 50 to 100 per cent. faster than glucose alone. The mechanism of this anomalous behaviour is discussed. It is possible that the capacity of this yeast to initiate the alcoholic fermentation of glucose is so much reduced that the cells in a pure glucose solution cannot utilise to their full extent the enzymes involved in the liberation of carbon dioxide and ethyl alcohol. By other yeasts, both glucose and fructose are converted at such a rate into a three-carbon intermediate compound that the latter is supplied in excess to the enzymic apparatus governing the last stages of alcoholic glycolysis. The bearing of the anomalies in alcoholic fermentation by Sauterne yeast upon other peculiarities in the metabolism of this micro-organism is being investigated.

P. H. P.

**Behaviour of Moulds on Expanded Corks.** J. Greger. (*Z. Unters. Lebensm.*, 1930, **60**, 532-536.)—The outer bark of the cork tree and the waste material from the manufacture of cork stoppers are used for the preparation of heat insulators, but may be infected with bacteria or moulds which, under favourable conditions, will develop subsequently and derive their sustenance from the nutrient matter carried in the cork. This is known as "primary infection," and treatment for 2 hours in air at 100° C. (or for 30 minutes at 150° C.) destroys the spores. Secondary infection may, however, occur subsequently, but a temperature of 260° C. for 10 minutes destroys the nutrient substances in the cork and ensures

complete sterilisation. Observations with *Mucor*, *Penicillium*, *Aspergillus* and *Dematium* are recorded. Sterilised cork soaked in water containing nutrient substances may become a source of secondary infection. J. G.

**Identification of Aspergilli in Cotton Goods.** G. Smith. (*J. Text. Inst.*, 1931, 22, T 110).—There are two main groups of *Aspergillus*, the *A. glaucus* series and the *A. penicilloides* (a series intermediate between *A. glaucus* and *A. fumigatus*, most of the strains resembling certain *Penicillia*.) A study has been made of 34 forms of mould of the group *A. glaucus*. They were grown on wort-agar at 25° C., the dimensions of the conidial apparatus were taken, the time required for the ascospores to ripen and the dimensions, markings, and colour of the latter observed. From these data, the forms were divided into four sub-groups: I, smooth without crests, ridges or furrow, green and yellow conidiophores (*A. repens* (Corda) Saccardo). II, smooth shallow furrow, no crests, intense red colour (*A. ruber*, Spieckermann and Bremer). III with furrow and small rounded crests (*A. amstelodami*, Mangin). IV with deep furrow and well-marked crests (*A. chevalieri*). The three species of the *A. penicilloides* series, recognised by Thom and Church, are diagnosed more fully and somewhat differently from the conceptions of the original authors. Eight plates are given, representing drawings and photomicrographs. R. F. I.

## Toxicological.

**Toxicology of Thallium.** G. Roche Lynch and J. M. S. Scovell. (*Lancet*, 1930, 219, 1340.)—The authors give details of three fatal cases of poisoning by thallium acetate, an accidental overdose of which was given for the treatment of ringworm. Other cases are recorded, in some of which the thallium salt had been used in form of a cream, to remove hair. Zelio corn and zelio paste, containing 2.1 to 2.8 per cent. of thallium, are extensively used in Germany as rat poisons, and have been responsible not only for accidental poisoning, but also for a number of criminal cases.

In the three fatal cases, referred to above, the thallium in the viscera and urine was determined as follows: A weighed quantity of the material was broken up with hydrochloric acid and potassium chlorate by the method of Fresenius and von Babo, and, when solution of all the material except the fat had been achieved, the liquid was filtered, the precipitate well washed, and the filtrate boiled until practically all the excess of chlorine had been driven off, or it was removed by the sulphur dioxide method. If necessary, it was filtered again, and the precipitate discarded. To the filtrate, ammonium chloride and ammonia were added, until it was distinctly alkaline, and it was then again boiled. The precipitate, consisting of iron, calcium, and magnesium, chiefly in the form of phosphates, was filtered off and discarded. The filtrate was then saturated with hydrogen sulphide, or an excess of freshly prepared ammonium sulphide was added; a black precipitate was formed. This was filtered off, well washed with dilute ammonium sulphide, and, finally, with distilled water. It consisted of

thallium sulphide ( $Tl_2S$ ), together with traces of other metals (copper) which are precipitated with hydrogen sulphide in alkaline solution. The precipitate, when completely washed, was dissolved off the paper with hot dilute hydrochloric acid. The thallium was converted into thallos chloride, any trace of copper which was present as the sulphide remaining behind. To the solution, ammonia was added in slight excess, and the liquid was boiled. Any precipitate was filtered off and discarded. The filtrate was then made very faintly acid with hydrochloric acid, and excess of potassium iodide solution was added. An immediate yellow precipitate of thallos iodide formed. As there is some tendency for thallos iodide to come down in colloidal form, the liquid was boiled and allowed to stand for 12 hours. The precipitate was then collected in a weighed Gooch crucible and well washed, first with potassium iodide solution, and subsequently with alcohol, until the washings gave no reaction for an iodide. The crucible and its contents were then dried at  $120^\circ C$ . until constant weight was obtained.

Although thallos iodide is very slightly soluble in water (1-17,000), it is almost completely insoluble in potassium iodide solution and in alcohol, so that filtration in the cold and washing with these reagents will give an accurate estimate of the amount present. The iodide, after weighing, may be confirmed as thallium by dissolving it in a solution of sodium thiosulphate, in which it is dissolved only with difficulty, whereas lead iodide is readily soluble. Finally, some of the iodide may be heated in a Bunsen flame on a platinum wire, and the characteristic green line in the spectrum obtained.

AMOUNTS FOUND IN THE VISCERA, EXPRESSED AS THALLIUM ACETATE.

Case A.					Case B.		Case C.		
Grains.					Grains.		Grains.		
Mgrms.					Mgrms.		Mgrms.		
Stomach	..	..	..	0.24	15.6	0.11	7.4	—	—
Liver	..	..	..	0.9	58.1	1.33	86.2	0.74	48.2
Intestines	..	..	..	1.4	90.8	1.73	112.8	0.23	15.0
Kidneys	..	..	..	—	—	0.12	7.9	0.09	6.0
Urine	..	..	..	—	—	—	—	0.28	18.2
Total	..	..	..	2.54	164.5	3.29	214.3	1.34	87.4
Amount given	..	..	..	39	2527	36	2333	28	1814
Amount intended to be given	..	..	..	3.9	253	3.6	233	2.8	181

B, who died first, had eliminated less of the drug than A, who was given a larger quantity.

From their experience and consideration of the literature (a full bibliography is given) the authors conclude that thallium acetate should not be used as an ordinary routine treatment for ringworm of the scalp, for the following reasons:—(1) Thallium in itself is a highly toxic substance, showing a marked similarity to lead, both in its chemistry and in its toxic symptoms, and the far-reaching effects of the poison are much greater than is generally supposed. (2) It has a definitely selective action on all forms of nervous tissue, and it has been



demonstrated that, even in infinitesimal doses, it causes slight degenerative changes in the brain cells of rats. It is, therefore, most unlikely to leave the human brain entirely unchanged, and it seems impossible to be certain that it does not hinder further brain development. (3) The margin between an epilating and a toxic dose is extremely small, and allows for no idiosyncrasies, whereas with X-rays the dosage is very accurate, trouble is rare, and if a mishap occurs it is at least local. (4) Ringworm of the scalp is not in itself a fatal disease, and, though often troublesome, can usually be cured by other means. It, therefore, does not seem justifiable to use such a powerful poison in an attempt to cure it a little more quickly. (5) Toxic phenomena do not appear to be due to the use of old solutions.

## Organic Analysis.

**Determination of Water in Glycerin.** L. F. Hoyt and P. C. Clark. (*Oil and Fat. Ind.*, 1931, 8, 59-61.)—The glycerin containing less than 10 per cent. of moisture was determined by a modification of the Bidwell and Sterling distillation method (*Ind. Eng. Chem.*, 1927, 17, 243), wherein toluene is used as the boiling liquid. It was found advisable to reverse the 45° slope of the tube leading from the boiling flask, to give a better flow back into the flask and more even distillation. For dynamite glycerin, 50 grms. or more, and for C.P. and crude glycerins 25 to 50 grms. are weighed to within 0.01 gm. into a 1 litre Pyrex flask, and 300 c.c. of toluene added. The graduated stem of the Bidwell-Sterling tube is filled with water-saturated toluene, and the flask is electrically heated until no more droplets of water come over. The bulk of the water comes over in the first half hour, and the last traces require 1½ to 2 hours. A small proportion of glycerin is carried over with the water, but the correction in no case exceeded 0.15 per cent. Commercial xylene, isopropyl ether and benzene were found unsuitable as boiling liquids. A dynamite glycerin, to which 10.37 per cent. of water had been added, gave by this method a recovery of 10.39 per cent. of water.  
D. G. H.

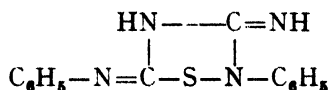
**Catechin of the Cacao Bean.** W. B. Adam, F. Hardy and M. Nierenstein. (*J. Amer. Chem. Soc.*, 1931, 53, 727-728.)—The authors have shown that the catechin isolated from cacao beans grown in Java is *l*-acacatechin,  $C_{15}H_{14}O_6$ , m.pt. 229° (which yields a penta-acetyl derivative, m.pt. 151°) and not a methyl derivative of catechin, as reported by previous workers. The optical rotatory power of this catechin is  $[\alpha]_D -69^\circ$  (in water), and that of its penta-acetyl derivative,  $[\alpha]_D -12^\circ$  (in tetrachloroethane). The same catechin was also isolated from cacao beans from West Africa and Trinidad. It is concluded that the catechin content of the cacao bean is about 0.8 per cent. Details of the method of isolation of the catechin are given.  
S. G. C.

**Chemical Properties of some Commercial Rayon Yarns.** B. P. Ridge, H. L. Parsons and M. Corner. (*J. Text. Inst.*, 1931, 22, T117.)—The materials investigated (first grade only) have included viscose, Lilienfeld, cuprammonium,

acetate and nitrocellulose rayons, and also purified chemically unchanged cotton, the following determinations being made: Copper number, loss of weight on boiling with alkali, methylene blue absorption, fluidity in cuprammonium hydroxide solution, ash content and ash alkalinity, material extracted by solvents, sulphur, copper and iron content, and (for acetate rayons) the acetic acid content. The methods of carrying out these determinations are given in full. Methylene blue absorption was carried out on the solution buffered to  $pH$  7. The test for fluidity in cuprammonium hydroxide solution was the same as described by Clibbens and Geake, except that the cellulose in the solutions was 2 per cent., instead of 0.5 per cent. in concentration. For the acetic acid content of acetate rayons the methods of Ost and of Barnett were used. In extracting with solvents, the acid-washed, rinsed and dried material was extracted with ether in one series, and with chloroform in another. The sulphur was determined by ashing the material with hydrochloric and nitric acids, the sulphate in the residue being precipitated and weighed as barium sulphate. The copper was determined by three methods: (a) direct precipitation as thiocyanate from a solution of the ash; (b) indirectly by measuring the catalytic power of a solution of the ash in accelerating the reduction of ferric salts by sodium thiosulphate; (c) colorimetrically, by means of diethyl-dithio-carbamate (Callan and Henderson). Iron was titrated with potassium dichromate solution, using diphenylamine as the indicator. The results are tabulated and very fully discussed. R. F. I.

## Inorganic Analysis.

**Detection of Carbon Disulphide.** F. Feigl and K. Weisselberg. (*Z. anal. Chem.*, 1931, 83, 93–104.)—The methods for the detection of carbon disulphide were re-investigated and compared. The most sensitive of the published tests is the precipitation of cuprous xanthate. Two new tests are described: (I) Hector's base,



is derived from phenylthiourea by oxidation with hydrogen peroxide. When a solution containing carbon disulphide is treated with the base and a few crystals of nickel acetate and gently warmed, a violet-red precipitate is produced, or a pink coloration, according to quantity. If the nickel salt is insoluble in the liquid to be tested, 5 to 10 drops of water are also added. The reaction is exceedingly sensitive, still more so if volatilisation is prevented by working in a closed tube. The precipitate has the composition  $\text{C}_{30}\text{H}_{22}\text{N}_8\text{S}_6\text{Ni}$ . (II) The formation of lead sulphide in an alkaline lead solution is considerably hastened by the presence of formaldehyde: one drop of the test solution, 2 to 3 of formalin, and one of plumbite solution are stirred together on a spot plate. A darkening occurs in a few seconds. If hydrogen sulphide is thought to be present, the drop to be tested is first treated with bromine water till yellow, after which a tiny crystal of sodium sulphite is

stirred in to effect decolorisation. Working with larger quantities in a test tube allows of the detection of smaller quantities of carbon disulphide. If the liquid is immiscible with water, a ring of lead sulphide will be formed at the zone of contact.

W. R. S.

**Cobaltic Sulphate as an Oxidising Agent.** S. Swann and T. S. Xanthakos. (*J. Amer. Chem. Soc.*, 1931, 53, 400-404.)—A study has been made of the preparation of cobaltic sulphate by electrolytic oxidation of cobaltous sulphate, and its use as an analytical reagent for the wet oxidation of certain organic compounds to carbon dioxide. For the preparation of cobaltic sulphate a saturated solution of cobaltous sulphate in 10 *N* sulphuric acid, contained in a porous cell which dipped into a beaker containing the same liquid, was oxidised anodically, using an anode of platinum sheet, a sheet of copper surrounding the porous cell serving as the cathode. A good yield of cobaltic sulphate was obtained by electrolysis for about 4 hours with an anode current density of 0.01-0.2 amp. per sq. cm., provided that the temperature was kept below 10° C. by cooling the electrolyte vessel in ice. The cobaltic sulphate was not isolated in the tests of its oxidising power on organic compounds; the suspension of the solid in sulphuric acid, which was the product of the electrolytic preparation, was used. The organic compound was stirred mechanically with an excess of this product for an hour at the ordinary temperature in a three-necked flask, which was fitted with an absorption train for carbon dioxide; air was then aspirated for half an hour through the apparatus, while the flask was heated on a water-bath. By this means it was found that, whereas amylenes, benzene and glycerin gave no carbon dioxide, and acetic acid, ethyl alcohol and acetone gave only a little, formic acid, tartaric acid, citric acid and malic acid gave the theoretical yield. Ethylene glycol gave a 96.05 per cent. yield of carbon dioxide. Formic acid remained unoxidised in the presence of amylene and glycerin.

S. G. C.

**Study of Spacu's Reaction. Volumetric Determination of Copper.** J. Golse. (*Bull. Soc. Chim.*, 1931, 49, 85-100.)—The author has studied Spacu's reaction (the precipitation of copper as cupric pyridine thiocyanate by the addition of pyridine and an alkali thiocyanate) with a view to making it the basis of a volumetric method of determination of copper. The methods proposed involve the precipitation of the copper by the addition of a regulated excess of the reagents, filtering off the precipitate, and determining the uncombined thiocyanate ions remaining in the solution, either (a) by precipitation by a known quantity of silver nitrate, the excess of which is determined by titration with standard ammonium thiocyanate, using iron alum as indicator; or (b) by oxidation with a known quantity of sodium hypochlorite, the unused excess of which is determined by addition of potassium iodide and titration of the liberated iodine with sodium thiosulphate. Even under the most favourable conditions which could be found, a certain proportion of the copper—about 2 per cent.—remained unprecipitated, and this necessitates the application of empirical corrections in the calculation of the amount of copper from the quantity of volumetric solution employed. The

methods, as so far worked out, cover the determination of copper in pure copper sulphate solutions only over a limited range of concentration, and they break down in presence of zinc, nickel, etc., owing to the pyridine thiocyanates of these metals being insoluble. On account of these limitations the details of the methods are not abstracted.

S. G. C.

**Separation of Beryllium from Aluminium. A. Travers and Schnoutka.** (*Compt. rend.*, 1931, 192, 285-287.)—The following modification of the method of Berthier was used for the separation of the two metals and the extraction of beryllia from beryl: the freshly-precipitated hydroxides are dissolved in excess of alkali, the solution saturated with sulphur dioxide and boiled for 10 minutes, and left in the cold for some hours. The alumina is quantitatively precipitated, occluding a little beryllia. The precipitate is, therefore, re-treated, when it yields nearly all the remaining ("*la presque totalité*") beryllia. The advantages claimed are, that the alumina precipitation is quantitative, and that a high yield is secured in the extraction of beryllia from the mineral. No numerical data establishing the completeness of the separation are given.

W. R. S.

**Rapid Colorimetric Estimation of Potassium. E. R. Caley.** (*J. Amer. Chem. Soc.*, 1931, 53, 539-545).—The following method is proposed for the determination of amounts of potassium between 0.01 and 0.001 grm.; it depends on the precipitation of potassium as picrate, which is separated, dissolved in water, and the yellow solution thus obtained matched colorimetrically: The solution containing the potassium as chloride (sulphates must be absent) is evaporated to dryness in a 25 c.c. beaker, the residue is dissolved in 1 c.c. of water, and 7.5 c.c. of a saturated solution of picric acid in 95 per cent. alcohol are added. The liquid is stirred until a precipitate forms, and kept for 40 minutes, with stirring at 5-minute intervals, the beaker being placed in a dish containing water at 20° C., to maintain a definite temperature during the precipitation. The potassium picrate is filtered off on a sintered glass filtering funnel having a medium porosity (filter paper will not do), and the precipitate is washed with successive 1-2 c.c. portions of ether until the washings are colourless. The ether having been removed by drawing air through the funnel, the precipitate is dissolved by pouring several successive quantities of water through the funnel, the solution being received in a 50 c.c. volumetric flask. The intensity of colour of the solution is compared in a plunger-type colorimeter with that of a standard solution of potassium picrate, containing practically the same amount of potassium as is being determined, prepared by submitting a measured volume of standard potassium chloride solution (1.907 grm. KCl per litre; 1 c.c. = 0.0010 grm. K) to the same procedure. This condition can be fulfilled only by preparing a range of standards, and choosing the one which is nearest to the unknown in colour for the final matching in the colorimeter; the standards keep well if protected from evaporation. The range of standards is called for to compensate for the solubility of potassium picrate. The method breaks down for amounts of potassium below 0.001 grm. Ammonium,

caesium and rubidium give insoluble picrates just like that of potassium. More than 0.007 grm. of sodium gave high results in the determination of 0.001 to 0.005 grm. of potassium. The presence of 0.005 grm. of lithium, magnesium, calcium, barium, strontium, aluminium or ferric iron caused no interference.

S. G. C.

**Decomposition of Refractory Silicates by Fused Ammonium Fluoride and its Application to the Determination of Silica in Glass Sands.** A. C. Shead and G. F. Smith. (*J. Amer. Chem. Soc.*, 1931, 53, 483-486.)—Berzelius noted the fusibility of ammonium fluoride, and Rose and Jannasch had independently employed ammonium fluoride for decomposing silicates, but, it is here suggested in such a way as to obscure the peculiar advantages of the reagent. The following process was tested with good results on two samples of glass sand, the silica content of which was known: The sample is thoroughly mixed with 5 grms. of crystalline ammonium fluoride in a weighed 25 ml. platinum crucible provided with a well-fitting lid. The crucible is placed in a hole through a heavy asbestos board so that about one-third of the surface of the crucible projects underneath. This part is maintained at a dull red heat for about 10 minutes. Experience showed that about 1 grm. of quartz (passing one hundred mesh sieve) should be volatilised within this time, but that a repetition of the process is necessary to complete the decomposition. Towards the end of the fume evolution, the burner is held in the hand with the flame impinging on the lid; this causes the detachment of the deposit of ammonium salt, which falls into the crucible, carrying with it any residue which may have been projected upwards during the decomposition. Before the crucible is allowed finally to come to redness, after the fluoride has been volatilised, it is recommended to add a drop or two of concentrated sulphuric acid, or a few fragments of ammonium persulphate, to prevent the escape of traces of aluminium or iron as halide. The residue is weighed, the loss in weight being due to silica.

S. G. C.

## Gas Analysis.

**Analysis of Mixtures of Hydrogen, Methane and Ethane.** O. J. Walker and S. N. Shukla. (*J. Chem. Soc.*, 1931, 368-370.)—The method depends on the removal of ethane by condensation (cf. *Trans. Faraday Soc.*, 1931, 27, 35), and of hydrogen by absorption on palladium. The apparatus consists of a Töpler pump connected through a 3-way tap to (1) a U-tube which may be cooled in a vacuum vessel with liquid air, and (2) a steam-jacketed palladium absorption-tube containing 3 grms. of palladium-sponge. Each of these 2 tubes is connected also to a train consisting of a mercury-trap, a transferring-pipette, and a bent capillary tube dipping into a mercury trough. The mixture (5 to 10 c.c.) is measured in a Bone and Wheeler burette and transferred, by means of a small tube inverted over mercury in a crucible, to the capillary tube at the end of the apparatus nearest the U-tube (1), and thence to the latter by manipulating the pump and the transferring pipette. After 5 minutes at the temperature of

liquid air the residual hydrogen and methane are removed and measured (the absorption giving the ethane), and passed to the palladium-tube, where they are heated for 20 minutes at 100° C. The steam is then replaced by cold water, and the residual gas measured to determine the hydrogen. A complete analysis takes 1½ hours, and the mean errors for mixtures containing ethane (10 to 45), hydrogen (31 to 64), and methane (16 to 50 per cent.) were  $\pm 0.8$ , 1.1 and 2.6 per cent., respectively. The percentage by volume found for any constituent seldom differs by more than 1 per cent. from the amount taken. Owing to the small volume of sample, no error arises from solution of methane in the condensed ethane (cf. Mulders and Scheffer, *Rec. trav. Chim. Pays Bas*, 1930, 49, 1057), but a check is provided if the methane and ethane are freed from hydrogen and exploded separately, and the ratio *contraction after explosion/contraction after absorption* in potassium hydroxide solution determined. This is essential if nitrogen is present.

J. G.

## Microchemical.

**Micro-Chemical Tests for Benzoic Acid, Salicylic Acid and Esters of *p*-Hydroxybenzoic Acid in Food and Drugs.** R. Fischer and F. Stauder. (*Mikrochem.*, 1930, 7, 330-338.)—The analysis is carried out on 1 to 2 grms. of material, such as jam, fruit juice, preserved vegetable, or syrup, which may contain 0.05-0.1 per cent. of the preservative to be identified. The material is acidified with hydrochloric acid, and, if the consistence is too thick, diluted with hot water. Fats are melted and digested with 2 to 3 c.c. of dilute sodium bicarbonate solution, and then the aqueous solution is poured off and acidified. The acid solution is extracted with a few c.c. of ether, and the ethereal solution is transferred, drop by drop, to a sublimation tube in a paraffin bath at a temperature not higher than 50° C. The different compounds are then separated by fractional sublimation, and the sublimates are identified by their crystalline form, their reactions, or melting points.

J. W. B.

## Reviews.

**QUANTUM CHEMISTRY.** By Prof. A. HAAS, Ph.D. Translated from the German by L. W. CODD, M.A. Pp. 75. London: Constable & Co., Ltd. 1930. Price 6s.

This book contains, in a somewhat extended form, the subject-matter of a series of four lectures that were delivered in 1929 before an audience which lacked both the time and opportunity for a comprehensive study of modern theoretical physics. In these lectures the recent views on atomic structure, based on the

principles of the quantum theory and wave mechanics, are given, and the scope of the book will be evident from the titles of the lectures, *viz.* (1) The Arithmetic of Chemical Periodicity, (2) The Quantum Theory of Valency and Chemical Forces, (3) Electron Grouping and the Periodic System, (4) Quantum Problems of Molecular and Nuclear Structure. Despite the highly mathematical nature of the subject, Professor Haas has succeeded in giving the gist of it without introducing mathematics. The book certainly achieves its object in providing a simple and lucid outline, and will, therefore, appeal to readers who desire to become acquainted with the modern trends of this branch of scientific knowledge. It also supplies an excellent introduction to the subject for more serious students.

As points in the book of special interest, reference may be made to the forecast and discovery of parahydrogen, and also to the calculations from theoretical considerations made by Gurney and Condon of the actual rate of radio-active disintegration.

In conclusion, it must be mentioned that the translation has been admirably done.

H. T. S. BRITTON.

PRACTICAL PHYSICAL CHEMISTRY. By ALEXANDER FINDLAY. Fifth Edition. Pp. xii+312. London: Longmans, Green & Co., Ltd. 1930. Price 7s. 6d.

This well-known work has undergone a real revision, and, owing to judicious excision, much new matter has been added without materially increasing its bulk. Contracted arithmetic has now been left for the school to teach before the student attempts physical chemistry. It is rather a pity that Prof. Findlay has not taken the opportunity afforded by the usual discussion of errors to explain what the "mean error," "mean square residual," or "standard deviation," as Pearson and Udney Yule (who prefer it to the "probable error") call it, really means. This is seldom made clear in works on probability; in fact, one sympathises with Lippmann's remark to Poincaré, apropos the fundamental theorem of the theory of errors, "Tout le monde y croit, parce que les expérimentateurs y voient un théorème de mathématiques, tandis que les mathématiciens le considèrent comme un fait d'expérience." A clear exposition of the meaning of the conventional expressions of this theory, so far as they apply to experimental work, would be really useful. As to the actual example, given on pp. 9-10, the subtraction of the mean 27.828 from its constituent 27.30 does not necessarily yield 0.003, although, as the third figure of that constituent is unknown, this is not impossible. A little algebra, applied to the formula given, will show how much more easily the precision of an average can be increased by greater accuracy of individual measurements than by the laborious method of repetition.

The author ignores the excellent work done in this country by the National Physical Laboratory on graduated apparatus; he adheres to the cubic centimetre, as against the millilitre, and does not mention the use of burettes with long graduations, which can be seen back and front, and so eliminate errors of parallax.

These are, of course, rather expensive for students' use; but, surely, were worth mentioning. He has abandoned the blowing-out method of delivery from pipettes and the use of the Shellbach burette.

The chapter on The Density of Gases and Vapours has been enlarged and is good, but there is too much rubber tubing in Fig. 21, where the two screw clips should be replaced by a two-way tap.

In the chapter on Viscosity and Surface Tension, the viscometer of Gibson and Jacobs, based on Stokes's law, is described. This good and simple instrument can be made and used by anyone who has a fairly exact centimetre scale, a thermometer and a few steel bicycle bearing balls, besides a stop-watch and the usual apparatus of the laboratory. The section on surface tension has been improved, but only the capillary tube and the drop method, both of which possess the merit of convenience, are described. The parachor is mentioned, and experiments on it are given. The rôle of the burette in the preparation of standard baryta solution (pp. 149-150) is not clear; a sentence seems to have dropped out.

It is now unusual to use the Lippmann electrometer in potentiometric work, but this is more or less a matter of taste. In this section on measurements of electromotive force, besides an adequate discussion of the electrometric method of determining hydron concentration, including Biilmann's quinhydrone electrode, there is some description of buffer solutions and their use in determining the pH value. The glass electrode, which is not very suitable for students' use, is not described.

The section on calorimetry is good, but it is a matter of taste whether a Beckmann thermometer, one of the German enclosed pattern, or one of the excellent solid-stem instruments which are made in this country, is used; in fact, the two latter are simpler to use, as the degree value does not need correction for varying temperature. It is unnecessary to give more than one standard substance for determining the water equivalent of a calorimeter, as benzoic acid is, by international agreement, used for this purpose, and its heat of combustion (based on Dickinson's work, which has been confirmed) is 6324 calories (15° C.) per grm. weighed in air. Naphthalene is a very unsuitable substance, and is not always easy to burn. The use of iron wire for firing a charge *seems* economical, but platinum with a cotton thread is better, as it does not injure the bomb lining, and there is no loss of platinum. The Regnault-Pfaundler correction formula is as simple as the graphic method given in this and other works, and is generally used by those who do much bomb calorimetry.

The reviewer has called attention to the above points, where, in his opinion, the book could be improved. He has been encouraged to do this by noting that some matters to which he called attention in the fourth edition have been altered in the fifth. The merits of the work are so outstanding in clarity of description, in the introduction of sufficient explanations of theory, and in copious references to literature, giving the student a chance of carrying his studies further, if an overcrowded curriculum allows him time to do this, that captious criticism, always



regrettable, is here impossible. It is a very useful book for the analytical chemist to have available for reference when some recondite problem seems a little outside the range of the usual analytical methods. It is to be hoped that before another eight years the author may give us another edition.

J. H. COSTE.

**THE CHEMICAL ANALYSIS OF ROCKS.** By HENRY S. WASHINGTON, Ph.D. Fourth Edition, re-written and enlarged. Pp. xi+296. New York: John Wiley & Sons, Inc. 1930. Price 20s.

This well-known hand-book has now reached its fourth edition, which may be taken as a measure of the well-deserved esteem in which it is held by the analytical fraternity. Its salient features are, or should be, by now so familiar that the reviewer need no longer give a sketch of the work. Suffice it to say that the operations of silicate rock analysis are described with a wealth of detail sufficient to enable a student or beginner, working by himself, to become competent in this class of work. The principal changes in the new edition refer to up-to-date appliances (such as Munroe and sintered glass crucibles), and special reagents (cupferron for titania, *o*-hydroxyquinoline for magnesia, and periodate for manganese); new text-matter on lithium and beryllium has been included.

The statement, on p. 274, that ignited beryllia "should always be dissolved in dilute hydrochloric acid and reprecipitated," is at variance with the general experience that the oxide is rendered insoluble in acids by strong ignition.

The author's technique in the bisulphate fusion of the ignited ammonia precipitate is very different from my own practice; a brief discussion of the operation may be of some practical interest. Pulped filter fibre is added to the precipitate (p. 173), so that a porous oxide results on ignition. This is subjected to fusion with 5 to 7 grms. of pyrosulphate (p. 180), which operation "can often be accomplished in three or four hours, especially if macerated paper has been used" (p. 182). The fusion is carried out in a platinum crucible.

Unless I am quite mistaken, the long time taken by the author over the fusion is due to the low temperature at which he conducts the fusion of a large quantity of pyrosulphate in platinum; raising the temperature in an attempt to hasten solution would be attended by great risk of loss due to foaming. This risk does not arise when a silica crucible is used.

The author, however, in common with Hillebrand and Lundell, rejects a silica crucible for bisulphate fusions, on the ground that "some silica may be taken up from it" (p. 178). If so, the amount is almost negligible, and can always be allowed for by a determination of the weight before and after fusion. The contamination with platinum is, in my opinion, a much more serious matter, the dissolved metal being more difficult to account for than silica; approximately, 0.0025 gm. of platinum is introduced into the analysis, "of which about one-half should be subtracted from the alumina" (p. 188).

A few unimportant misprints were noticed. There is one, however (p. 178, line 23), to which attention should be called: the loss in weight of a vitreosil crucible is not "0.02 to 0.04 gram" for a bisulphate fusion, but 0.0002 to 0.0004 gm. The author, whose attention I called to the statement as it appears in print, has requested me to rectify it in the sense indicated.

W. R. SCHOELLER.

RECENT ADVANCES IN ANALYTICAL CHEMISTRY. Vol. I: ORGANIC CHEMISTRY.  
Editor: C. AINSWORTH MITCHELL. Pp. 421. London: J. & A. Churchill.  
1930. Price 12s. 6d.

From time to time there arises the necessity for some considered statement of the advances made in chemical subjects. To some extent annual reports published by various bodies meet the need, but more than this is required by those not actually engaged in investigating processes for themselves. The volume now considered is an attempt to supply critical summaries of organic analytical chemistry. There are eleven chapters, each written by well-known workers on the subject under discussion. It can be stated at once that the objects aimed at, namely, to enable the reader to ascertain how far he can displace his present methods for new ones with safety, is in the main achieved.

The chapter on sugar analysis by Mr. Hinton is a very full discussion of analytical methods. He calls attention to the utility of invertase in place of acid hydrolysis of sucrose, and deals adequately with the ratio of ash and conductivity of sugar solutions. He states—rightly, in the reviewer's opinion—that the conductivity should be accepted on its own merits, for it is not safe to assume that the soluble inorganic impurities are always the same in sugar solutions. The various copper methods for determining reducing sugars receive full treatment, and the value of Lane and Eynon's use of methylene blue as an internal indicator is stressed. McLachlan's method of examining malt extract and glucose by the use of various types of yeast is likewise discussed in some detail.

Chapter II, by Dr. Ainsworth Mitchell, is a careful discussion of present-day methods of oil analysis. In view of the fact that there is a tendency to discount the utility of "iodine" values in investigating the chemistry of fats and oils, it is satisfactory to note that the use of halogen absorption methods still holds the field. Bolton and Williams's classification of oils into groups by means of iodine values of the unsaponifiable matter is here given suitable prominence. Kaufmann's thiocyanogen absorption method receives due attention, and its importance for determining the character of unsaturated bonds is indicated briefly. The work of Armstrong and Hilditch on the oxidation of unsaturated fatty acids provided a new mode of attack of glycerides in two directions: (1) Determination of the proportion of saturated and unsaturated glycerides, and (2) the nature of the unsaturated fatty acids in the glycerides. This enabled Hilditch to deal with the question of the distribution of fatty acids in the glycerides of seed fats.

Dr. Mitchell also contributes a chapter on tannins, which deals especially with recent methods for the analysis of tanning materials, galls, and tannin-bearing materials of all kinds. Mitchell's quantitative method for determining pyrogallol tannins by means of ferrous tartrate, first described in 1923, and his separation of gallic acid from gallotannin by means of alkaloids, are discussed in detail. The method described here is convenient and rapid for the purpose. Recent gravimetric processes for tannin estimations receive adequate treatment, and the utility of precipitation by alkaloids is critically examined in this chapter.

Mr. Parry contributes the chapter on essential oils, and he calls attention to the two valuable reports issued by the Committee of the Society. The more recent work on determinations of ionone, carvone, citral, alcohols and phenols in essential oils, is described with clearness. The utilisation of magnesium methyl iodide in evaluating essential oils for their content of hydroxy compounds is stated to be complicated for ordinary use, but it does not appear so to the reviewer, for the technique of the preparation of the reagents is easy to work with the materials available to-day. A useful account of the methods of detecting addition of esters is given as fully as the subject calls for, particular attention being devoted to ethyl laurate, an adulterant which is sometimes used. Parry gives an account of some tests carried out by himself on the determination of ascaridole, the main constituent of American wormseed oil, the results of the tests leading to the adoption of an iodometric method based on a factor developed from determinations with as pure ascaridole as was available.

Dr. Dorothy Jordan Lloyd provides a chapter on the proteins, giving in detail the carbamate method for the analysis of gelatin and describing some of the new units obtained by hydrolysis and carbamation. Methods for the determination of tyrosine, tryptophan and cystine are dealt with in the light of recent work, and a short account of enzyme action as applied to protein analysis is included in the chapter. Methods of electro-dialysis, with illustrations of apparatus, find a place in this section. Dr. Lloyd's conclusion is sufficiently significant to be quoted:

"In conclusion, it is important to notice that in considering the value of proteins as food materials for the living (and particularly the growing) animal, the knowledge obtained by a chemical analysis does not carry the matter very far forward. While certain units must be present in a "good" food protein, the availability of any unit for absorption and synthesis is not indicated as yet by any known method of analysis. For the assay of the value of a protein from the biological standpoint, recourse must still be had to biological methods."

This leads naturally to the account of the biological analysis of proteins by Dr. Harriette Chick, who supplies a brief description of the methods for ascertaining the biological value of a protein.

The section on Cereals by Dr. D. W. Kent-Jones should be studied, if only for

its introductory paragraph and penetrating enquiry into the utility and scope of the various determinations suggested or made on cereals from time to time. Typical sections illustrating the method adopted by Dr. Kent-Jones in his discussion are those on moisture, protein distribution in flour, gluten (see particularly the first three lines on page 226), sugar and colour. One cannot but feel that the kind of criticism applied in this chapter is desirable for methods of analysis in many other fields of work. "Milk and Milk Products" are dealt with by Mr. Elsdon, who considers critically the determinations usually carried out on milk, and also sets out the methods of the Committee of the Society on condensed milk. Hortvet's modification of Monier-Williams's method for determining the depression of the freezing point of milk for detection of added water is examined and recommended. It is well to emphasise the fact that sour milk is not suitable for testing by the method (page 252). If any comment is called for on this chapter, it is solely on the ground of its being too short.

Mr. Dickson's chapter on paper analysis is full, and calls for little comment. The special section on the use of polarised light in the examination of fibres is largely the outcome of Mr. Dickson's own experience, and is a valuable aid to the chemist who has to examine paper critically. Recent methods for determining the nature and proportions of fillers and sizing are furnished for general use, and a number of machines for physical testing is described. A simple readily constructed apparatus for air porosity is depicted (page 300), which appears to be quite suitable for practical purposes. The method for determining tar acids in special antiseptic wrapping papers (page 291) is likely to give seriously low results with phenol and the cresols, for it is known that the rejected aqueous layer contains appreciable quantities of these substances.

Two chapters are assigned to petroleum and coal, both written by Dr. King and Mr. Acton-Taylor, jointly. A general description of elementary analysis, as applied to oils, forms the first part of these chapters, with a useful commentary on the value of the methods of ascertaining moisture and aromatic hydrocarbons. A specially useful section is that dealing with viscosity, some of the more novel methods or applications of older ones receiving attention. It is satisfactory to note that the tendency to use absolute methods for determining viscosity is a feature of the recent technical investigations here discussed. In the chapter on coal the statement that some methods still remain which are not fully approved (page 350) is one with which the reviewer is in entire agreement. Even such determinations as that of phosphorus in coal are not yet completely worked out in all cases. For arsenic the most serious source of error is the method of preparing the solution for estimating the element, and not so much the actual separation of the arsenic recovered.

Although the volume is entitled "Organic Analysis," a very valuable chapter on Gas Analysis is included, written by Mr. Ambler. The methods for the detection and determination of the constituents of gases in large or small proportions are, as would be anticipated, given with due regard to their importance or probable

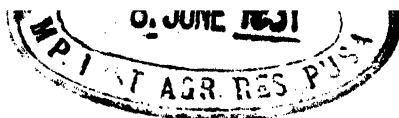
utility in practical testing. Thus the sections on carbon monoxide, hydrogen and methane, and on fractional combustion processes are important critical compilations on these subjects. The sole remark called for on this chapter is that Mr. Ambler has probably been somewhat reticent in stating his opinion on some of the methods proposed in the literature, particularly on some of the "rapid" methods.

In the opinion of the reviewer this volume is a valuable work of reference on recent analytical methods. It furnishes an illustration of the fact that analytical chemistry offers a field for research of increasing importance in its application to pure chemistry and to technological practice.

J. J. Fox.

## Publications Received.

- BIOASSAYS. A HANDBOOK OF QUANTITATIVE PHARMACOLOGY. By JAMES C. MUNCH. London: Baillière, Tindall & Cox. Price 45s. net.
- INDUSTRIAL MICROBIOLOGY. By H. F. SMITH and W. L. OBOLD. London: Baillière, Tindall & Cox. Price 27s. net.
- A MONOGRAPH OF VISCOMETRY. By GUY BARR. Oxford University Press. Price 30s. net.
- TECHNICAL METHODS OF CHEMICAL ANALYSIS. 2nd Ed. Vol. III. By G. LUNGE and C. A. KEANE. London: Gurney & Jackson. Price £3 3s. net.
- A LABORATORY MANUAL OF ELECTROCHEMISTRY. By E. MÜLLER. Translated from the 4th Edn. by H. J. T. ELLINGHAM. London: Routledge. Price 15s. net.
- RECENT ADVANCES IN PHYSICAL AND INORGANIC CHEMISTRY. By A. W. STEWART. 6th Edn. London: Longmans, Green & Co. Price 18s. net.
- CHEMICAL ENGINEERING AND CHEMICAL CATALOGUE, 1931. London: Leonard Hill. Price 15s.
- BRITISH CHEMICALS AND THEIR MANUFACTURERS, 1931. Association of British Chemical Manufacturers. Gratis to genuine users of chemicals.
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# THE ANALYST

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## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

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AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, April 1st, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—George Brown, A.I.C., Charles Loudon, B.Sc., A.I.C., Charles Percy Money, B.Sc., F.I.C., Martin Priest, F.I.C., Arthur Goodyear Simpson, M.A., and Gerrish Smith.

Certificates were read for the second time in favour of:—K. N. Bagchi, B.Sc., M.B., D.T.M., William Nelson Bradshaw, B.Sc., Adrian Joseph Clifford Lickorish, F.I.C., Ernest Grenville Purser, B.Sc., A.I.C., and William Waddell Robson.

The following were elected Members of the Society:—Cecil Chilvers, B.Sc., F.I.C., Jack Hubert Hamence, M.Sc., A.I.C., Cecil John House, B.Sc., A.R.C.Sc., F.I.C., and Henry George Rees, B.Sc., A.R.C.Sc., A.I.C.

The following papers were read and discussed:—"Carbon Monoxide Poisoning: Its Detection and the Determination of the Percentage Saturation in Blood, by means of the Hartridge Reversion Spectroscope," by R. C. Frederick, A.I.C.; "Experiments on the Hardness of Fats," by H. M. Mason, M.Sc., F.I.C., and G. Walsh, B.Sc., A.I.C.; "A New Process for the Determination of Small Amounts of Bromide in Chloride," by B. S. Evans, M.C., Ph.D., F.I.C.; and "The Use of Bromine as a Reagent in the Determination of Alkaloids," by S. G. Walton and R. G. O'Brien.

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### NORTH OF ENGLAND SECTION.

A JOINT meeting of the Section with the Yorkshire Analysts' Association was held at Leeds on April 25th.

The following papers were read and discussed:—"Some Aspects of the Bacteriological Examination of Water," by W. G. Carey, F.I.C.; and "River Pollution Prevention Problems," by J. W. H. Johnson, M.Sc., F.I.C. A discussion on Sandalwood Oils was introduced by J. R. Walmsley, F.I.C.

There was an attendance of thirty, including the President (Dr. J. T. Dunn). The Chairman (Mr. C. J. H. Stock) presided.

## NORTH OF ENGLAND SECTION.

ADDRESS OF THE CHAIRMAN (MR. G. D. ELSDON),

*Delivered at the Meeting held at Manchester, February 14th, 1931.*

AT the meeting of the Committee of this Section, held on December 6th, 1930, it was decided that the Annual Meeting should include among its agenda an address to be given by the Chairman. I was very strongly in favour of this suggestion being put into effect during succeeding years, but just as strongly opposed to it during the present session. There were two reasons, however, which caused me to consent. Firstly, I thought that it might be a very favourable opportunity to place on record the circumstances surrounding the formation of this section; and, secondly, that it would form a precedent for future holders of the office who would, from year to year, be able to give helpful and stimulating addresses to the members.

With the approval of the Council, a letter was sent on May 13th, 1924, by Mr. S. E. Melling, to all members of the Society living more than about 30 miles north of Birmingham, in order to see what demand there was for a North of England Section. The response to this letter was so encouraging that an extraordinary General Meeting of the Society was held in the rooms of the Manchester Literary and Philosophical Society on November 7th, 1924, at which very many London members were present. At this meeting it was resolved that a North of England Section should be formed, and I hope I may be forgiven for adding that I shall always remember with pride and pleasure that I acted as the first Hon. Secretary of the Section, although only in a temporary capacity. The first meeting of the Section was held on Saturday, February 14th, 1925, in the Milton Hall, Manchester, at which the rules of the Section were adopted, after full discussion, and Prof. W. H. Roberts, Messrs. J. Wood and H. T. Lea were appointed Chairman, Vice-Chairman and Secretary, respectively, together with a Committee consisting of Messrs. Elsdon, Hurst, Melling, Ross, and Stubbs.

The first ordinary meeting was held on Saturday, June 27th, 1925, at the Queen's Hotel, Leeds, and from that time the Section has continued to be successful. The meetings have always attracted an excellent attendance, considering the comparative smallness of our numbers and the very considerable distances which many of the members have to travel. This success has been due to two main reasons, the help and encouragement which has always been given by the Council of the Society, and the keenness of the members themselves. I feel that each one of those who have attended our meetings can take to himself some share of the success which has been attained. That this success has also been a source of advantage to the members themselves I think no one will deny. By mutual, helpful criticism, by debate, by opportunities of social intercourse, we have widened our outlook and been helped in the solution of our difficulties. I would here urge those of our younger members who may have such difficulties to bring them freely before the meetings or before other members privately, in the full knowledge that those who have special information and long experience will be glad to lend a helping hand to those not so happily placed.

The past session, which is brought to a close by this meeting to-day, has been one of the most successful of all. We have now held five meetings during which seven original papers have been presented and two discussions on general topics have been introduced, quite apart from a considerable number of smaller points which have been raised during the progress of the meetings. It is a most fitting

finish to an excellent session that our Secretary has been able to prevail upon Prof. T. P. Hilditch to give us the benefit of papers by himself and his co-workers, which we are shortly to have.

Apart from the general progress which has taken place in our profession during the year, there is one point of special interest both to Public Analysts and to works' chemists and manufacturers, on which perhaps I might say a few words. I refer to the publication of a series of Jam Standards by the Food Manufacturers' Federation. In certain quarters these standards have been subjected to a considerable amount of adverse criticism, particularly on the point that the Society has taken some part in the negotiations. This is not the place to engage in any controversy concerning the merits or demerits of the suggestions put forward. Scarcely anyone, I imagine, will claim that they are beyond criticism; but one can, at least, say that the decisions were reached after very full and careful examination of all the points which are at present being brought against them, and that they are the expression of a sincere desire to grapple with a most difficult situation.

The question of Food Standards is one which is surrounded by difficulties on every side. Only a few years ago the majority of Public Analysts and nearly all manufacturers were definitely opposed to them. More recently there has been a change in outlook, and the idea is gradually gaining ground. In my own opinion the institution of standards will do good. It should certainly remove from the market all those articles which are definitely inferior, whilst those makers who have gradually built up a trade in a high-class article, based upon a reputation gained over many years, are not likely to risk decrease in or loss of their trade by lowering their standard to any minimum which is proper. It may be argued that neither the Society nor a combination of manufacturers is suitable for such work, and this may be true, but until such time as our rulers find time to take this subject to themselves it would appear that our energies might more reasonably be given to the making of constructive proposals, than to the adverse criticism of those who are making every effort to improve the present state of our food supply.

Of special interest to many of our members was the Cheese Bill which was considered by Parliament during the year, but which was unfortunately crowded out owing to pressure of other business. Almost everyone approved of this Bill in principle, although some of the standards suggested were open to criticism. It is to be hoped that opportunity may be given during the coming year for a re-introduction of a similar Bill, and that it may have a more successful career than the former one.

The subject of milk continues to occupy a large proportion of the time of many of our members. It is now 30 years since the publication of the report of the Departmental Committee, but the passage of time has not served to settle the differences of opinion which the report induced. Among much that seems to be controversial there must be, at least, some points upon which even our most sceptical members are in agreement, and it seems to me to be of the utmost importance that these points of agreement should be thoroughly explored, so that each member may know exactly where he agrees with and differs from, the remainder of his colleagues.

In this connection there would appear to be a somewhat lamentable lack of desire upon the part of Government Departments to avail themselves of the assistance which could be afforded to them by our Society. Two important points have recently come to my knowledge. The Minister of Agriculture has intimated that an Inter-Departmental Committee has asked Dr. Tocher to deal with the available milk data along the lines of his "Variations in the composition of milk,"



and has asked certain persons to send any data which they have to Dr. Tocher. The Empire Marketing Board has apparently set up a committee dealing with the freezing-point of milk, and has a report ready for publication. They have also asked certain persons to use the apparatus which they have designed to determine the freezing-point of milks to be supplied. Neither the Ministry nor the Board has seen fit to approach the Society as a whole, and it would appear at least remarkable that the majority of those who are most intimately connected with these subjects should be in complete ignorance of the action which is being taken.

It would be impossible for me to close these few words without giving some expression of my appreciation of the honour which you have paid me in electing me as your Chairman for the past year. As far as I know I have only one qualification for the office, a great love for our profession together with a keen desire that the Society and our Section may prosper. In your kindness you have magnified this to such an extent that you have paid me this honour, an honour which I am not likely to forget. I feel that the year has been a most successful one, a fact of which I can speak without diffidence, as this success has been so largely due to the unremitting efforts of our most excellent Honorary Secretary, Mr. J. R. Stubbs, and the unqualified support which the members themselves have given in the orderly conduct of meetings.

I should further like to express the satisfaction with which the committee regard the support which the Section has had from its younger members. Its future must largely rest in their hands, but, judging by the enthusiasm which they are now showing, we need have no fear for its continued success.

## Deaths.

WITH great regret we record the deaths of the following Members:-

G. J. Alderton, on March 10th, 1931.

M. Wynter Blyth, on March 23rd, 1931.

## The Determination of Laevulose in Sweetened Condensed Milk.

BY C. L. HINTON, F.I.C., AND T. MACARA, F.I.C.

FOR the purpose of finding the proportion of true milk constituents in condensed milk, it is desirable that satisfactory methods should be available for determining the amounts of any sugars other than sucrose or lactose. For example, the determination of any laevulose that might be present would be of value. Appreciable amounts of this sugar would come either from invert sugar intentionally added or from the inversion or breakdown of sucrose in the sample after manufacture; and in either case, the analysis of the sample upon ordinary lines, showing only lactose and sucrose, would lead to an erroneous interpretation of the results. The present

paper describes a rapid method for the direct determination of laevulose in condensed milk.

The method depends essentially upon a principle originally due to Kolthoff (*Chem. Weekblad*, 1922, 19, 1; *ANALYST*, 1922, 47, 301), and recently elaborated by Kruisheer (*Z. Unters. Lebensm.*, 1929, 58, 268). This consists in the oxidation of aldose sugars—in this case lactose, and dextrose if present—with alkaline iodine solution, and subsequent determination of the unchanged laevulose by copper reduction. We have modified Kruisheer's procedure so as to secure the conditions most satisfactory for condensed milk. Other slight modifications would no doubt enable the method to be adapted for other products.

To avoid the interference of sucrose in the copper reduction which occurs with Fehling's solution, Kruisheer used the copper solution of Luff, a citrate-carbonate reagent, the use of which has been revived by Schoorl (*Z. Unters. Lebensm.*, 1929, 57, 566; *Brit. Chem. Abst.*, 1929, B, 952). This solution is reduced by sucrose to a negligible extent.

In determining the amount of copper reduced, we have departed from Kruisheer's procedure, which depended on a volumetric determination of the unreduced copper. It seemed more desirable to determine the cuprous oxide itself, and for this purpose we have adopted the convenient and delicate process of Shaffer and Hartmann (*J. Biol. Chem.*, 1921, 45, 349; *J. Chem. Soc.*, 1921, 120, ii, 417), which does not require any separation of the cuprous oxide from the excess of cupric salt.

The stages of the process, then, are as follows:

- (a) *Preparation of the serum.* This is carried out according to either of the methods described in the Society's "Report on the Determination of Sucrose" (*ANALYST*, 1930, 55, 111).
- (b) *Oxidation of the lactose* (and dextrose, if present) by treatment with alkaline iodine solution.
- (c) *Reduction of copper (in Luff's solution) by the laevulose.*
- (d) *Determination of reduced copper* by the iodimetric method of Shaffer and Hartmann.

The last three stages can be completed in 30 minutes.

We give below a description of the process in detail in its final form, and add a few notes describing certain matters that were dealt with in working it out.

#### SPECIAL SOLUTIONS REQUIRED.

Sucrose solution: approximately 9 grms. per 100 ml. (freshly prepared).

Normal iodine solution: 13 grms. of iodine and 15 grms. of potassium iodide per 100 ml.

Sodium carbonate and hydroxide solution: a mixture of approximately equal parts of 2 *N* sodium carbonate and 2 *N* sodium hydroxide solutions.

Sulphuric acid: approximately 5-normal.

Sodium sulphite solution: 20 per cent.

Sodium sulphite solution, 2 per cent.: freshly prepared or diluted from the stronger solution.

**Luff's solution:** dissolve 25 grms. of copper sulphate crystals in 100 ml. of water; 50 grms. citric acid in 50 ml. of water; 368 grms.  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$  in 300 or 400 ml. of lukewarm water. Add the citric acid solution to the sodium carbonate solution, and then add the copper solution. Mix, cool, make up to 1 litre and filter.

**Iodate-iodide solution:** 2.7 grms. of potassium iodate, 80 grms. of potassium iodide, and 10 ml. of 0.5 *N* sodium hydroxide solution per litre.

**Potassium oxalate solution:** a saturated aqueous solution.

**Sodium thiosulphate solution:** approximately 0.05 *N*, accurately standardised (but see Note 5).

**PROCEDURE.** (a) *Preparation of the Serum.*—As mentioned above, this may be prepared by clarification with either zinc ferrocyanide or phosphotungstic acid as described in the "Report on Sucrose." If the latter method is used, the serum should be carried forward to its further treatment within thirty minutes of the clarification, in order to avoid significant inversion of the sucrose.

A control serum should also be prepared from fresh milk (100 ml. of milk with the same quantities of precipitants as for the condensed milk, made up to 200 ml. with water and filtered).

(b) *Oxidation of the Aldose Sugars.*—Place 10 ml. of each serum in 250 ml. conical flasks, taking care that the liquid does not flow on to the sides of the flask. To the fresh milk serum add 10 ml. of 9 per cent. sucrose solution, and to the other 10 ml. of water.

To each then add 5 ml. of *N* iodine solution and 6 ml. of the 2 *N* mixed alkali solution, shake round, and allow the flasks to stand for 10 minutes (in a bath at 15–20° C. if the room temperature is very low). Acidify with 1.6 ml. of 5 *N* sulphuric acid, and remove the liberated iodine first with 20 per cent. sodium sulphite solution, and finally, after adding five or six drops of starch solution, with 2 per cent. sodium sulphite solution. (This operation should have the precision of a titration, though the quantities need not be measured.) Immediately afterwards add 1 drop of methyl orange solution and neutralise with 2 *N* sodium hydroxide solution (or with the mixed alkali solution).

(c) *Treatment with Luff's Solution.*—Add 20 ml. of Luff's solution to each flask, heat the contents to boiling in about 2 minutes on a plain wire gauze over a burner, then attach the flask to a reflux condenser, resting it on asbestos-covered gauze, and boil gently by heating with a Bunsen flame. (The burner, suitably regulated, should be already in position beneath the gauze when the flask of boiling solution is transferred to it.) After exactly 10 minutes' boiling, remove the flask and cool in running water for four or five minutes.

(d) *Titration of Reduced Copper.*—Add exactly 25 ml. of the iodate-iodide solution, and 20 ml. of saturated potassium oxalate solution. Acidify carefully, while swirling, with 20 ml. of 5 *N* sulphuric acid. Shake round (with some care, owing to frothing) until the precipitate of cuprous oxide (partly converted into white cuprous iodide) has dissolved, then titrate with 0.05 *N* thiosulphate solution. No further starch should be required. The end-point should be a sharp change to a fine light-blue.

**CALCULATION OF THE LAEVULOSE.**—The difference between the titrations of the sample and of the control, as ml. of 0.05 *N* thiosulphate, multiplied by 0.064, gives the percentage of laevulose in the sample, uncorrected for the effect of the volume of clarification precipitate. The volume of the precipitate can be calculated and allowed for as described in the "Report on the Determination of Sucrose."

It is preferable to determine the exact value of the thiosulphate in terms of laevulose by making a test on 10 ml. of the fresh milk serum with a known amount of added invert sugar. (The latter may be used in the form of a solution of 0.5 per cent. or stronger, so that not more than a few ml. need be added to the serum.)

**RESULTS OBTAINED ON KNOWN AMOUNTS OF LAEVULOSE.**—A series of determinations was carried out, by the method described, on portions of a sample of condensed milk to which known amounts of invert sugar were added. The mixtures were clarified after the addition of the invert sugar. It will be seen that a small amount of laevulose was indicated in the condensed milk itself, when compared with the fresh milk control. The amounts of laevulose found have been corrected for the effect of the volume of precipitate.

Laevulose found.

Laevulose added. Per Cent.	Total. Per Cent.	Less amount in sample. Per Cent.
0	0.03	—
0.05	0.07	0.04
0.10	0.13	0.10
0.25	0.29	0.26
0.50	0.53	0.50
0.75	0.77	0.74
1.00	1.01	0.98

**NOTES.**—(1) At the end of stage (b), if the acidified solution, after removal of the iodine, is not neutralised at once, there is a risk of some inversion of the sucrose. Thus, in control tests on the fresh milk serum with added sucrose, one was neutralised within 2 minutes of acidification, another after standing for 8 minutes. The solutions were then carried through the remainder of the process as usual, and showed the following differences from a blank carried out with no sucrose present:

Neutralised within 2 minutes.	Laevulose equiv. to 0.15 ml. <i>N</i> /20 thiosulphate.
„ after 8 „	„ „ 0.7 ml. „ „

An increase in the amount of acid above that specified will result in further risk of inversion. The combined effects can cause fairly large errors. When an extra 1.5 ml. of acid was added (*i.e.* double the amount necessary), and the mixture was left for 8 minutes before neutralising, laevulose equivalent to 6 ml. of *N*/20 thiosulphate was found. (This would correspond with an apparent 0.4 per cent. of laevulose in the sample.)

It is most important, therefore, that the procedure laid down should be closely followed, so as to avoid this source of error.

(2) The Luff's solution should be prepared carefully according to the formula given, otherwise the precipitation of the cuprous oxide is unsatisfactory. The titration of the reagent against standard acid is advisable as a check; 10 ml. should require about 45 ml. of 0.5 *N* acid, with methyl orange as indicator. The 20 ml. of Luff's solution specified in the method should not be varied, otherwise difficulties may be encountered in dissolving the cuprous oxide precipitate, or in securing a satisfactory end-point. This reagent is very sensitive, and should only be used under standard conditions.

(3) The alkalinity of the solution during the iodine oxidation of stage (b) was the cause of some difficulty at first. In the earlier experiments, 2 *N* sodium hydroxide alone was used as the alkali, and the amount necessary to ensure complete oxidation of the lactose had to be controlled very closely. Thus, in tests on fresh milk serum, with no sucrose or invert sugar added, the following series of results was obtained for varying amounts of added alkali:

Amount of 2 <i>N</i> sodium hydroxide. ml.	Final thiosulphate titration. ml.	Difference from blank. ml.
Blank (reagents only)	34.5	
3.0	33.0	1.5
3.3	34.35	0.15
3.4	34.5	0.0
3.5	34.4	0.1
3.6	34.2	0.3
3.7	30.5	4.0

The differences represent unoxidised lactose, so that a variation of a fraction of a ml. of alkali solution might cause appreciable errors in estimations of laevulose. In later experiments, it was found that the optimum amount of alkali was not always the same. For one milk serum examined it was, for instance, in the neighbourhood of 3 ml., an amount which gave incomplete oxidation in the earlier case. Extending the time of oxidation was tried with excess of alkali present, but did not give complete oxidation.

Owing to these difficulties, plain sodium hydroxide solution was abandoned in favour of a weaker alkali, consisting of a mixture of sodium carbonate and sodium hydroxide. This gave much more satisfactory results, as the optimum range was less restricted. Thus, the following were the titrations obtained in a series of tests with varying amounts of the mixed alkali:

Amount of mixed alkali. ml.	Final thiosulphate titration. ml.
3	32.0
4	36.75
6	37.1
8	35.3

This and other tests showed that 6 ml. of the mixed alkali was a safe amount to adopt for general cases. The amount of acid for acidification was modified to correspond with the change in the amount of alkali.

(4) The milk is clarified by the zinc ferrocyanide method; the ammonia used prior to the clarification gives, on the addition of the iodine and alkali, a black precipitate of nitrogen iodide, which partly disappears during the subsequent standing. This phenomenon seems to have no effect, however, on the laevulose determination. In parallel experiments the same thiosulphate equivalent was found for the laevulose whether ammonia was used or not.

(5) The factor for converting the thiosulphate differences to laevulose is the mean of the values obtained in two series of experiments, in which known amounts of invert sugar solution were added in one case to fresh milk serum, in the other to condensed milk serum. Ammonia was used in preparing the serum.

The process was carried out as described in this paper, the invert sugar solution being added, in the form of a 0.50 per cent. solution (*i.e.* 0.25 per cent. laevulose) to the 10 ml. portions of serum taken for the test. The results are shown in the following table. The amounts of laevulose, shown as added to the fresh milk serum under I, are expressed as if they were the corresponding percentages present in a sample of condensed milk, a 50 per cent. serum from the fresh milk being taken to be approximately equivalent in composition to a 20 per cent. serum from the condensed milk.

	Laevulose added. Per Cent.	Titration N/20 thiosulphate. ml.	Difference, due to laevulose. ml.	Factor for converting to per cent. of laevulose.
I. Fresh milk serum.				
	0	36.8	—	—
	0.25	33.0	3.8	0.066
	0.5	29.0	7.8	0.064
	0.75	25.0	11.8	0.064
	1.0	21.3	15.5	0.065
			Mean	0.065
II. Condensed milk serum.				
	0	36.0	—	—
	0.25	32.0	4.0	0.063
	0.5	27.95	8.05	0.062
	0.75	24.1	11.9	0.063
	1.0	20.4	15.6	0.064
			Mean	0.063

From these data, a mean factor of 0.064 was taken. Clearly this gives the percentage as it would be indicated if there were no volume of precipitate correction. The latter, therefore, has to be allowed for in the usual way.

As before mentioned, it is preferable to establish the thiosulphate equivalent for the laevulose (added in the form of invert sugar solution) alongside the test on the sample, and its control, rather than to rely on the stated factor. This procedure automatically eliminates any errors due to slight differences in manipulation, and avoids the necessity for exact standardisation of the thiosulphate.

(6) It is important that the iodine and alkali solutions be reasonably close to the strength specified, otherwise the oxidation of the lactose may not proceed satisfactorily.

(7) Some doubt was felt at first as to the adequacy of the conditions for oxidising the lactose, if there should be appreciable amounts of dextrose present and if the room temperature at which the oxidation was carried out were too low. Experiments showed, however, that the laevulose could be determined correctly up to 1.5 per cent. when added as invert sugar (*i.e.* in conjunction with a like amount of dextrose), and at a room temperature as low as 10° C.

(8) Attempts were made to give added accuracy to the process by doubling the amount of serum worked with. This, however, was found to be definitely a disadvantage. The amounts of the various reagents used had to be correspondingly doubled, and this appeared to be responsible for cuprous deposits which adhered to the bottom of the flask, and were with difficulty dissolved by the iodine. The conditions given in the method as adopted seem to be the most satisfactory for securing a cuprous oxide precipitate which dissolves readily.

The above method was developed in connection with the work of the Milk Products Sub-Committee, at a time when it was considered that appreciable quantities of invert sugar might be found in sweetened condensed milks after fairly lengthy storage. As the result of applying this method to the analysis of some milks it has been discovered, however, that, while invert sugar was undoubtedly formed, the laevulose had been apparently converted simultaneously into laevan, which has no reducing action, only small amounts (about 0.5 per cent.) of laevulose remaining as such. No direct reduction method will therefore indicate the full percentage of sucrose which has been inverted. Further, the determination of sucrose by polarimetric methods is affected, as laevan has a specific rotation which is considerably less negative than that of laevulose, but it is hydrolysed to laevulose by the usual process of inversion.

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## The Detection of Small Quantities of Calcium.

By NORMAN EVERS, B.Sc., F.I.C.

*(Read at the Meeting, December 3, 1930.)*

THE work described in the present paper was the outcome of an investigation into the possibility of including in the next edition of the British Pharmacopoeia a simple test which would be generally applicable for detecting traces of calcium in the salts described therein. It was desired that such a test should be capable of being used as a limit test, so as to lay down definite limits of calcium where this element was likely to occur.

Calcium is most likely to occur in magnesium carbonate and oxide, so that any method suggested must be capable of application to these compounds. Current methods for the detection of small quantities of calcium in magnesium salts are most unsatisfactory. For this reason the United States Pharmacopoeia (X) gives a long and tedious quantitative test for calcium in magnesium carbonate and oxide, depending on a double precipitation as oxalate. The present edition of the British Pharmacopoeia is content with stating that "no reaction" or "not more than the slightest reaction" for calcium should be obtained.

**PRECIPITATION AS OXALATE.**—Although complications were naturally expected when dealing with magnesium salts, it was thought that the oxalate precipitation would probably be applicable to other salts, and the first experiments were made to investigate this point. It soon became apparent that this test was quite useless for the purpose. The influence of salts on the precipitation was far too great.

The following experiments give an indication of the results obtained:

A control test was carried out with 2 mgrms. of calcium, 1 c.c. of dilute ammonia solution and 1 c.c. of 2.5 per cent. ammonium oxalate solution in 50 c.c. of solution. In the absence of salts a turbidity was produced almost immediately.

The experiment was repeated, adding 1 gram. of a number of salts, with the following results:

Adding 2 mgrms. of calcium.

*Added Salts.*

No added salt.  
Sodium chloride, 1 gram.  
Borax, 1 gram.  
Sodium potassium tartrate, 1 gram.  
Potassium citrate, 1 gram.

*Result.*

Almost immediate pptn.  
Slight ppt. after 3 hours.  
Slight ppt. after 3 hours.  
No ppt.  
No ppt.



Adding 5 mgrms. of calcium.

*Added salts.*

No added salt.  
Sodium chloride, 1 grm.  
Borax, 1 grm.  
Sodium potassium tartrate, 1 grm.  
Potassium citrate, 1 grm.

*Result.*

Immediate pptn.  
Almost immediate pptn.  
Slight ppt. after 30 minutes.  
Slight ppt. after 30 minutes.  
No ppt.

Variations in the concentration of the reagents did not appreciably improve matters. It was found that even 0.25 grm. of potassium citrate in 50 c.c. of solution prevented the precipitation of 2 mgrms. of calcium.

Further complications would be introduced if magnesium were also present in the salt as an impurity. This line of investigation was therefore abandoned.

**CALCIUM OLEATE TEST.**—The formation of an opalescence on the addition of sodium oleate solution to a solution is an extremely delicate test for calcium. Under the best conditions 0.01 mgrm. of calcium in 50 c.c. of solution, or 0.00002 per cent., can just be detected.

The test is also, of course, a test for magnesium, but is much less sensitive, 0.6 mgrm. in 50 c.c. of solution, or 0.0012 per cent., being the minimum quantity which can be detected. Further, within certain limits of concentration the precipitation of magnesium is entirely suppressed in the presence of potassium citrate, whilst the sensitiveness of the calcium test is actually increased.

The best conditions for the detection of calcium were found to be as follows: Take 50 c.c. of the solution containing calcium, which should be neutral or slightly alkaline. Dissolve in it 2 grms. of potassium citrate, and add 0.3 c.c. of a solution prepared by dissolving 10 grms. of oleic acid in 200 c.c. of 1 per cent. sodium hydroxide. Mix and allow the mixture to stand.

An excess of the reagent gives less opalescence. A certain excess of alkali is desirable for the best results. The test is only satisfactory between certain limits of calcium concentration. With quantities exceeding 1 mgrm. in 50 c.c. the opalescence is actually reduced. Under the above conditions quantities of magnesium up to 15 mgrms. give no opalescence.

Summarising the results, the oleate test is excellent for quantities of calcium varying from 0.01 mgrm. up to 1 mgrm. in the absence of more than 10 mgrms. of magnesium, and within these limits in the absence of other salts the opalescence appears proportional to the calcium present.

Further experiments showed, however, that, in spite of its delicacy, the oleate test is not suitable for the purpose in view. Possibly, if the test could be carried out, using standards containing the same concentration of the same salt, it would be satisfactory, but this is hardly practicable.

The addition of other salts, even in the absence of potassium citrate, caused the results to be erratic. This was partly due to their "salting out" effect on the soap, which sometimes caused flocculation, but this was not the whole explanation.

**THE SULPHATE METHOD.**—The differing solubilities of the sulphates, particularly in dilute alcohol, appeared to offer possibilities, and a search of the literature was made with this object in view.

Crookes ("Select Methods of Chemical Analysis," p. 52) states that, according to Scheerer, satisfactory results are obtained "by converting the alkaline earths into neutral sulphates, and adding alcohol to the aqueous solution until a persistent cloudiness is produced. After some hours all the calcium sulphate is deposited. When too much alcohol has been used some of the magnesium sulphate is deposited as well."

Hundeshagen (*Z. öffentl. Chem.*, 1909, 15, 85), in a method for the analysis of magnesite, suggests that, after obtaining the mixed chlorides by evaporation with hydrochloric acid, the residue should be treated with 30 c.c. of warm water and 4 grms. of sodium sulphate, and the solution mixed with 40 c.c. of 90 per cent. alcohol. After standing for four hours the precipitated calcium sulphate is filtered off, washed with 50 per cent. alcohol, and dissolved in hydrochloric acid, being then precipitated as oxalate in the ordinary way.

Preliminary experiments were carried out with calcium alone in the presence of excess of dilute sulphuric acid adding twice the volume of 95 per cent. industrial methylated spirit.\* The calcium was added as  $N/5$  calcium chloride solution. As the test was primarily intended for testing magnesium oxide and carbonate, it was considered that it would be more convenient if it could be carried out in the presence of an excess of sulphuric acid.

A quantity containing 2 mgrms. of calcium with 25 c.c. of 10 per cent. sulphuric acid and 50 c.c. of 95 per cent. alcohol was found to become turbid after standing about 20 minutes, and after half-an-hour a flocculent precipitate separated. In the presence of 2 grms. of pure magnesium sulphate a similar precipitate was formed, but the turbidity appeared somewhat sooner.

More detailed tests were then carried out for the purpose of discovering the limits of the test and the best conditions.

**Limit of Magnesium Concentration.**—Five grms. of magnesium sulphate or 1 gm. of pure magnesium oxide deposited crystals of  $MgSO_4 \cdot 7H_2O$ , but 3 grms. of magnesium sulphate and 0.5 gm. of magnesium oxide remained clear. These latter quantities or their equivalents in other magnesium salts should, therefore, not be exceeded.

**Concentration of Sulphuric Acid.**—Increase of concentration of sulphuric acid was found to make the test more sensitive up to 20 per cent., but above this concentration the turbidity was reduced.

**Concentration of Alcohol.**—Reduction of the amount of alcohol below double the volume of dilute sulphuric acid reduced the sensitiveness of the test. Increasing quantities of alcohol were inadvisable owing to crystallisation of magnesium sulphate.

\* Referred to subsequently as alcohol.

*Sensitiveness of the Test.*—Two grms. of pure magnesium sulphate, with the required amount of calcium as  $N/5$  calcium chloride solution, were dissolved in 25 c.c. of 20 per cent. sulphuric acid, and 50 c.c. alcohol were added.

Calcium. Mgrms.	Time of precipitation.
0	No ppt. in 24 hours
0.1	Faint trace of ppt. in 24 hours
0.2	Trace of ppt. in 24 hours
0.4	Slight ppt. in 24 hours
1	Turbidity in 25 minutes
2	Turbidity in 6 minutes
4	Turbidity in 2 minutes
8	Turbidity immediately

The test therefore appears capable of detecting 0.1 mgrm. of calcium in the presence of 0.2 mgrm. of magnesium, or a Mg : Ca ratio of 2000:1.

*Effect of Magnesium Concentration.*—Differing quantities of magnesium sulphate were taken, 1 mgrm. of calcium being added to each.

Magnesium sulphate. Grms.	Calcium. Mgrms.	Turbidity time. Minutes.
3	1	15
2	1	20
1	1	30
0.5	1	45
0	1	60

A sample of calcium-free magnesium oxide gave similar results.

It is desirable, therefore, to keep the magnesium concentration within narrow limits.

*The Qualitative Test.*—The following is the final form of test adopted:—Dissolve the required weight of magnesium salt in 25 c.c. of 20 per cent. (by weight) sulphuric acid, and add 50 c.c. of 95 per cent. alcohol. Note the time at which a definite turbidity first appears. If none appears in half-an-hour, less than 1 mgrm. of calcium is present.

*Commercial Samples.*—A sample of light magnesium oxide gave the following results:

Magnesium oxide. Grm.	Turbidity time.
0.25	Immediate
0.2	5 minutes
0.15	23 minutes
0.1	60 minutes

This sample, therefore, contained about 1 mgrm. of calcium in 0.15 gm. = 0.66 per cent. of calcium.

The results on other commercial samples are shown in the following table:

Samples of.	Number tested.	Weight taken. Grm.	Number clear after 30 mins.	Number turbid after 30 mins.
Magnesium oxide, light	6	0.2	3	3
		0.1	6	0
		0.4	6	0
Magnesium oxide, heavy	2	0.2	2	0
Magnesium carbonate, light	8	0.5	2	6
Magnesium carbonate, heavy	7	0.25	8	0
		0.5	6	1
		0.25	7	0

These samples were from three different manufacturers in this country. With one exception, the "heavy" varieties contained very little calcium, but the "light" varieties were much more frequently contaminated.

*Quantitative Method.*—The above test has the disadvantage that it depends on individual judgment as to whether a solution is turbid or not. As a rough test, it may be regarded as satisfactory, but more definite results may be obtained by weighing the calcium sulphate after allowing the precipitate to stand overnight. The results obtained are slightly low, as calcium sulphate appears to possess a small solubility in the solution. The test was carried out in the same way as before, taking 2 grms. of magnesium sulphate and adding definite amounts of calcium, but experience showed that the use of 25 per cent. sulphuric acid, instead of 20 per cent., gave slightly higher results. After standing overnight the precipitate was filtered off on a Gooch crucible previously washed with alcoholic sulphuric acid and ignited. The residue was washed with 200 c.c. of a mixture of 2 volumes of alcohol and 1 volume of 25 per cent. sulphuric acid, ignited, and weighed as calcium sulphate. The following results were obtained in the presence of 2 grms. of magnesium sulphate in each case.

Calcium taken. Mgrms.	Calcium found. Mgrms.	Error. Mgrm.
0.4	0.3	—0.1
1.0	0.8	—0.2
2.0	1.5	—0.5
3.0	2.6	—0.4
5.0	4.5	—0.5
10.0	9.2	—0.8
20.0	18.9	—1.1

The agreement between duplicate determinations was within 0.1 mgrm. in each case. The results are obviously somewhat low, but, considering the convenience of the method, its use as a limit test for calcium in magnesium carbonate and oxide should be quite satisfactory.

*Effect of Chlorides.*—One and a half gram. of calcium-free magnesium chloride, to which 2 mgrms. of calcium had been added, gave 5.2 mgrms. of calcium sulphate = 1.5 mgrm. of calcium, so that the presence of chlorides has little effect on the result.

Commercial samples of magnesium oxide and carbonate from three British manufacturers gave the following results:

		Taken. Grm.	Calcium sulphate. Mgrms.	= Calcium oxide. Per Cent.
Magnesium carbonate, light ..	A	0.5	6.6	0.54
	B	0.5	5.0	0.41
	C	0.5	7.2	0.59
	D	0.25	3.2	0.53
Magnesium oxide, light ..	A	0.2	4.0	0.82
	B	0.2	10.6	2.18
	C	0.2	3.4	0.70
	D	0.1	2.2	0.91
Magnesium carbonate, heavy	A	0.5	Nil	—
	B	0.5	Nil	—
	C	0.5	Nil	—
	D	0.5	1.8	0.15
Magnesium oxide, heavy ..	A	0.2	Nil	—
	B	0.2	Nil	—
	C	0.2	Nil	—

Three samples of light magnesium carbonate containing stated amounts of calcium were kindly supplied by the Washington Chemical Company. The results were:

Calcium oxide stated. Per Cent.	Taken. Grm.	Calcium sulphate weighed. Grms.	= Calcium oxide. Per Cent.
Less than			
0.1	0.5	0.1	0.01
0.2	0.5	1.8	0.15
0.6	0.5	7.2	0.59

A reasonable standard would appear to be that not more than 10 mgrms. of calcium sulphate should be obtained from 0.5 gm. of magnesium carbonate or 0.2 gm. of magnesium oxide, corresponding with a maximum of 1.9 per cent. of calcium oxide in the oxide and 0.8 per cent. in the carbonate.

The method has also been tried tentatively in other cases where the oxalate method presents difficulties. It has been found that the results are unaffected by the presence of iron and phosphates, and the method has been used without any difficulty for the determination of calcium in such a preparation as compound syrup of iron phosphate or chemical food, in which the calcium determination is otherwise a troublesome business.

I wish to acknowledge the assistance of Mr. C. E. Davis in the experimental work.

## The Detection of Benzoic Acid.

By ALFRED NORMAN LEATHER, B.Sc., F.I.C.

*(Read at the Meeting of the North of England Section, December 6, 1930.)*

THE following method for the detection of benzoic acid (and, incidentally, salicylic acid) is especially applicable to jams, sauces, cordials, mineral waters and milks, but is of fairly general application to foodstuffs and beverages. A slight modification supplies a means of detecting with certainty the presence of a benzoyl radicle in an organic compound when only a very small quantity (1-2 mgrms.) of the compound is available. The method depends on the distillation of benzoic acid with steam, in the apparatus described, into a solution of alkali in such a way that the volume of the latter is not materially increased. Benzoic acid is detected by an application of an observation by Hinks (ANALYST, 1913, 38, 555), that an ethereal solution of benzoic acid becomes cloudy on adding a drop of strong ammonia solution. As steam-volatile acids other than benzoic yield a turbidity in this reaction, the presence of benzoic acid is confirmed by the formation of basic ferric benzoate, or, in some cases, of characteristic crystals of silver benzoate.

The process may be completed in twenty minutes, and, in the form described, will detect 100 parts of benzoic acid per million.

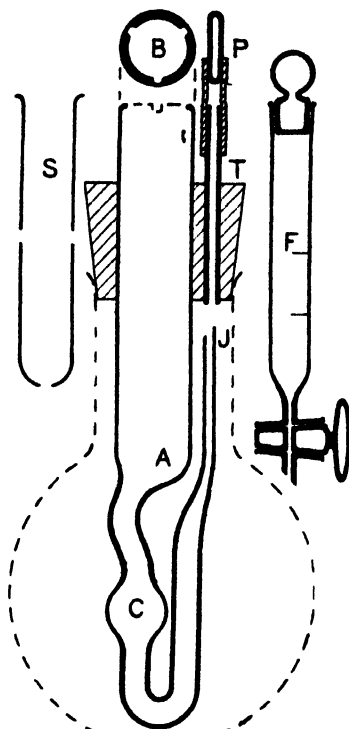
**APPARATUS.\*** The apparatus shown in the diagram (half the actual size) consists of a 250 ml. round, flat-bottomed, wide-mouthed flask ("CO<sub>2</sub>" flask), fitted with a rubber stopper carrying a special U-tube (A). The rubber stopper must be easily removable. It has two holes, one to take a tube 2 cm., and the other a tube 4 mm. in diameter. Through the larger hole passes the widened limb of the U-tube, which reaches almost to the bottom of the flask. The widened portion is 10 cm. long, and at its upper end has a slight rim turned inwards with three indentations in it. A plan-sketch of this end is shown at (B). The U-bend itself is made from glass tubing having an internal diameter of 5.5 mm. Between the wide limb and the U-bend is a bulb (C), 1.5 cm. in diameter, the centre of which is 3 cm. above the bottom of the U-bend. Above the bulb the tube has a slight outward curve. The other limb of the U-tube is drawn out, about 5 cm. above the U-bend, to an internal diameter of 2 mm., and this drawn-out limb reaches up to within 6 mm. of the stopper, where it is cut off at an angle of 45° (J). The volume of liquid required to fill the U-tube up to the level of the bottom of the bulb is approximately 1 ml., while 0.5 ml. of liquid is sufficient to "seal" the U-bend.

A thin-walled tube (S), 7.5 cm. long and 1.4 cm. in external diameter, and having a number of holes (3 mm. in diameter) in the bottom and sides, passes down inside the wide limb of the U-tube, and rests with its rim on the inward-turning rim of the latter. This perforated tube acts as a splash-guard, and must hang clear of the walls of the U-tube.

\* The Scientific Glass-Blowing Co., 12, Wright Street, Burlington Street, Manchester, can supply the complete apparatus for 7s. 6d.

Through the other hole of the rubber stopper passes a glass tube (T) flush with the lower surface of the stopper, and extending about 2 cm. above the upper surface. This tube carries at the upper end a short piece of rubber tubing which may be closed by a glass plug (P).

A small cylindrical separator (F) has a capacity of 4 ml., and is fitted with a glass stopper and capillary tap (1.5 mm. internal diameter). The capillary tube



above and below the tap should be short. Marks are made on the separator indicating a content of 1 ml. and 2 ml. above the tap.

**DESCRIPTION OF THE TEST.**—Transfer 10 grms. of the sample to the flask, add 75 ml. of saturated brine, 1 ml. of 10 per cent. sulphuric acid, and about 0.5 gm. of coarsely powdered pumice. Fit the rubber stopper (carrying the U-tube) into the flask. Drop 0.5 ml. of approximately *N* sodium hydroxide solution into the mouth of the tube so that it runs down into the U-bend, pass the splash-guard (S) down into position, and close the rubber tube with the glass plug (P). Place the flask on a thin iron gauze over an ordinary Bunsen flame and boil for three minutes. The weight of steam driven out of the apparatus should be about 10 to 12 grms.

The brine aids the distillation of benzoic acid (*cf.* Monier-Williams, *Public Health Reports*, No. 39, Ministry of Health). The steam passes through the U-tube and comes into intimate contact with the sodium hydroxide solution. The

upper part of the tube is designed to allow the steam to pass out and return the splashings to the bulb. Since a large portion of the U-tube is immersed in steam or boiling liquid, little condensation occurs. (The volume of liquid in the U-tube is somewhat increased by condensation just as boiling is commencing; afterwards some evaporation occurs and the volume slowly diminishes.)

Remove the apparatus from the flame and take out the glass plug (P). Remove the rubber stopper and U-tube from the flask, and rinse the outside of the U-tube with a jet of water from a wash-bottle. Lift out the splash-guard (S), allowing the drop of liquid adhering to it to drain back into the U-tube. Incline the U-tube in such a way that the contents run out of the end (J) of the drawn-out limb, and collect the liquid as completely as possible in the small separator (F).

To the liquid in the small separator add two drops of 50 per cent. sulphuric acid and about 1 ml. of ether (using the marks on the sides as a guide). Shake thoroughly and run off the lower layer. Wash the ether by shaking with an equal volume of water. After washing, the volume of ether is slightly reduced. Again wash the ether with a correspondingly reduced volume of water, and repeat the washing a third time. Run off the water as completely as possible. Add one drop of strong ammonia solution (from a "quill" tube). If benzoic acid is present in the sample to the extent of 100 parts per million, the ether becomes turbid.

If a turbidity forms, it is necessary to apply the following confirmatory test:— Shake the separator thoroughly, and run off as completely as possible the small aqueous layer into a hollow microscope-slide and evaporate just to dryness by warming on a steam-heated plate (*e.g.* by putting the slide across a corner of a covered water-bath). Cool the slide and dissolve the residue in a drop of water (conveniently added by means of a platinum wire loop about 5 mm. in diameter). Add at the edge of the drop (by means of a loop about 3 mm. in diameter) a small drop of ferric alum solution (7.5 per cent., filtered). The presence of benzoic acid is confirmed by the formation of a zone of the opaque buff basic ferric benzoate precipitate.

**SALICYLIC AND CINNAMIC ACIDS.**—If these substances are present in the sample, they distil in the same way as benzoic acid. They are extracted by ether in the small separator, and cause a turbidity when the drop of strong ammonia solution is added, provided that they are present in the sample to the extent of 100 parts per million. They yield residues of ammonium salicylate and cinnamate, respectively, when the drop of ammoniacal extract is evaporated on the hollow slide. On testing the residue with ferric alum, as described, ammonium salicylate yields an intense blue-violet zone. The ammonium cinnamate residue is not completely soluble in water (probably owing to partial hydrolysis during evaporation). The drop, however, containing some few undissolved particles, yields a precipitate with ferric alum of ferric cinnamate resembling the benzoate in texture, but yellow instead of buff in colour. If to the same drop a further addition is made in the cold of a small drop of purple alkaline permanganate (2.5 per cent. sodium hydroxide, 2.5 per cent. potassium permanganate) on a 5 mm. loop, immediate



reduction to a green colour occurs, and an odour of benzaldehyde is noticeable. (*Distinction from benzoic acid.*)

**SULPHUR DIOXIDE.**—If sulphur dioxide is present in the sample it distils in the same way as benzoic acid, and is transferred to the small separator. It should here be oxidised by the addition of slight excess of potassium dichromate solution (one or two drops of a concentrated solution), after acidification, and before extraction with ether. If not removed, it dissolves in the ether, from which it is not all removed by washing, and it yields a turbidity with ammonia.

**OTHER ACIDS GIVING THE TURBIDITY REACTION.**—I have found that an ethereal solution of almost any ether-soluble acid yields a turbidity on adding a drop of strong ammonia solution. In order to interfere with the test an acid must fulfil the following conditions:—It must be volatile with steam; it must be soluble in ether; it must not be removed from ether by washing with water under the conditions of the test.

These conditions are fulfilled by the whole series of volatile fatty acids from butyric upwards. However, these acids do not usually occur in foodstuffs in the free state in sufficient quantity to give a turbidity in the test. Some exceptions are: Rancid butter, very sour milk, certain vinegars and preparations therefrom.

When the drop of the ammoniacal solution of the turbidity due to these acids is evaporated on the hollow slide almost to dryness, the odour of the free acids is noticeable. The residue does not usually completely re-dissolve on adding the drop of water. On adding the drop of ferric alum a turbidity may be produced, but this may be seen to be due to the separation of oily drops, and is readily distinguishable from the benzoate precipitate.

Slight modifications are recommended when applying the test to the following:

(a) *Vinegar, Pickles and Sauces.*—It is necessary to increase the amount of caustic soda solution in the U-tube to prevent the liquid becoming acid before the end of the three minutes' boiling. It may be necessary to use 1 ml. of 10 per cent. caustic soda solution.

(b) *Mineral Waters and Wines.*—Twenty ml. of the sample may be used, in which case the turbidity reaction is obtained if the sample contains 50 parts per million of benzoic acid. If it is desired to detect 10 parts per million, proceed as follows:—Shake 200 ml. of wine, acidified with 1 ml. of 10 per cent. sulphuric acid, with 100 ml. of ether. Run off the wine and shake the ether with 10 ml. of 2 per cent. sodium hydroxide solution. Run off the alkaline layer, acidify with sulphuric acid, transfer to the apparatus and complete the test as described.

(c) *Fats.*—Fats tend to retard the distillation of benzoic acid with steam. If the time of boiling is increased to six minutes, the turbidity reaction may be obtained if the sample contains 100 parts per million of benzoic acid.

(d) *Flour.*—Benzoic acid in the proportion of 20 parts per million has been detected as follows:—Shake thoroughly 50 grms. of flour with 150 ml. of ether, and filter into a separator. Shake the ether with 10 ml. of 2 per cent. sodium

hydroxide solution containing 20 per cent. of alcohol. Run off the alkaline layer, acidify with sulphuric acid, transfer to the apparatus, and complete the test as usual, but increase the time of boiling to six minutes.

**A MICRO-REACTION FOR BENZOIC ACID.**—Instead of testing the residue of ammonium benzoate on the hollow slide with ferric alum as described above, a very sensitive and distinctive reaction may in most cases be obtained with

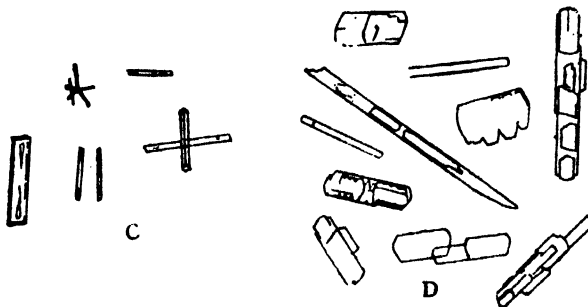


SILVER BENZOATE  $\times 20$ .

A. Ordinary light.

B. Polarised light.

silver nitrate. The residue on the slide is dissolved in a small drop of water in the cold, and a minute particle of solid silver nitrate is added to the centre of the drop on the tip of a platinum wire. Very small crystals are formed at first,



TYPICAL FORMS  $\times 40$ .

C. Silver benzoate.

D. Silver salicylate.

close to the place where the silver nitrate was added. After a minute or two, larger and more characteristic crystals appear (see photographs and sketch). These are best observed by polarised light. Where a crystal is free from superimposed crystals, it may be shown to exhibit parallel extinction. The crystals are very thin and appear chiefly gray and white between crossed Nicols. Under

similar conditions ammonium salicylate yields well-formed and readily distinguishable crystals of its silver salt (see sketch). These consist of long, comparatively thick, four-sided prisms, which exhibit brilliant colours between crossed Nicols.

The presence of volatile fatty acids interferes with the formation of the characteristic crystals described. The ammonium benzoate residue recovered from jams, cordials and mineral waters readily gives characteristic crystals of silver benzoate, but that from pickles and sauces containing vinegar (which contains traces of butyric and higher acids) and from coffee extract and rancid butter may fail to give them.

**A TEST FOR THE BENZOYL RADICLE, USING ONLY 1 TO 2 MGRMS. OF THE COMPOUND.**—Hydrolyse the compound by suitable treatment (*e.g.* by heating in a small test-tube with a drop of alcoholic potassium hydroxide) and transfer to the flask of the apparatus described. Add 25 ml. of brine and 1 ml. of 10 per cent. sulphuric acid, together with about 0.5 gm. of coarsely powdered pumice. Complete the test as usual, but increase the time of boiling to 5 minutes. In general, no noticeable turbidity occurs on adding the drop of ammonia to the small separator. A small residue is obtained on the slide if the compound contains the benzoyl radicle. Dissolve the residue in a very small quantity of water, and confirm by adding a particle of silver nitrate and observing the characteristic crystals of silver benzoate.

PUBLIC ANALYST'S DEPARTMENT,  
PUBLIC HEALTH LABORATORY, MANCHESTER.

## Investigations into the Analytical Chemistry of Tantalum, Niobium, and their Mineral Associates.

### XIX. Laboratory Notes on Analytical Technique.

By W. R. SCHOELLER, Ph.D.

(*Work done under the Analytical Investigation Scheme.*)

THIS Section should be grouped with Section VI (ANALYST, 1926, 51, 613), as it deals with another phase of the specialised technique gradually evolved in the course of this research. Whilst the earlier paper discusses processes for dissolving the earth acids after *alkali* fusion and recovering them from such solutions, the present one describes the treatment of solutions resulting from fusion with an *acid* flux (*i.e.* bisulphate). This brief laboratory companion should assist those interested in the subject; at the same time, avoidance of repetition will simplify the text of subsequent Sections.

Certain improvements of recent date are here given for the first time. The manipulations will be discussed in the following order: *A*, Bisulphate fusion. *B*, Solution of bisulphate melt; destruction of tartaric acid. *C*, Recovery of earth acid from tartrate solution. *D*, Recovery from oxalate solution. *E*, Filtration and washing of precipitates. *F*, Ignition and purification of precipitates. *G*, Micro-tests for earth acids.

**A. BISULPHATE FUSION.**—A platinum crucible should not be used for this operation, for two reasons. First, the fusion is a protracted one, because any attempt to hasten it by increasing the heat results in considerable foaming, with dissipation of sulphur trioxide and risk of loss. Secondly, the fused mass becomes contaminated with platinum, which interferes in subsequent separations. A silica glass or vitreosil crucible is free from these objections. In contact with the non-metallic surface, the bisulphate does not foam at all readily when rapidly heated to the dissociation temperature; hence the fusion can generally be accomplished in less than ten minutes, the crucible being manipulated with the tongs throughout.

Potassium bisulphate is more convenient and effective than the sodium salt, which loses sulphur trioxide at a lower temperature. If the flux becomes pasty before the attack is complete, it is regenerated by heating with about 0.5 c.c. of strong sulphuric acid. The attack on the silica crucible is practically negligible: thus, one crucible of silica glass, which had already been in use for a long time before being put under observation, lost 0.0052 grm. after 32 fusions, an average of 0.16 mgrm. per fusion. Another crucible of the same make suffered no loss in weight after the first three fusions.

**Procedure.**—The empty silica crucible is weighed before and after fusion; an allowance for silica can then be made, if necessary. The proportion of flux is about 3 grms. for 0.25, 4 for 0.5, 6 for 1 grm. of material. The bisulphate need not necessarily be dehydrated by a preliminary fusion before the material is added: when an ignited oxide is fused for re-treatment, the flux is added to it and gently heated on an asbestos mat till fused; any loss by dusting during the transfer of the oxide is thus obviated. The crucible is then held over the free flame, gently rotated from time to time, and the heat raised till the fused mass is clear. The mass is made to solidify in a thin layer around the sides of the crucible; 0.5 c.c. (or more) of strong sulphuric acid is added, and the fusion repeated. The melt is allowed to set clear of the bottom, the crucible being supported in an inclined position.

**B. SOLUTION OF BISULPHATE MELT; DESTRUCTION OF TARTARIC ACID.**—Two solvents are in use: a saturated solution of ammonium oxalate, and 20 per cent. tartaric acid (roughly the same weight of solid reagent as the bisulphate taken).

To remove the cake from the crucible, the latter is partly filled with the solvent, placed on a pipeclay triangle, and gently heated with a moving flame at the point where the melt adheres; as a rule the lumps slide off the side of the crucible almost at once. The crucible contents are transferred to a beaker; the crucible is thoroughly rinsed, first with hot solvent, then distilled water, heated, and weighed.

The liquid in the beaker is stirred and warmed till the cake is dissolved. With ammonium oxalate no difficulty will be experienced, but tartaric acid lixiviation may prove troublesome with mixed oxides in which tantalum largely preponderates; no doubt the solution of tantalic oxide is assisted by mineral associates such as niobium, titanium, and iron. If they are absent or very subordinate, a certain amount of hydrolytic precipitation of tantalic acid often takes place, with formation of cloudy or opalescent colloidal solutions. Now it is essential, at least for the precipitation of members of the hydrogen sulphide group, to secure a clear solution of the fused mass. To achieve this when dealing with materials of high tantalum content, the operator has the choice of two procedures.

(1) The bisulphate melt is leached in the crucible (capacity, 50 c.c.) with cold 20 per cent. tartaric acid solution with the assistance of air agitation. The melt disintegrates, leaving a pulverulent residue, which dissolves when the liquid is heated on the water-bath.

(2) The bisulphate melt is extracted with ammoniacal ammonium tartrate (a 20 per cent. solution of tartaric acid containing an excess of 10 c.c. of strong ammonia). The tartaric earth-acid complexes being more stable in ammoniacal than in acid solution, ammonium tartrate is a better solvent for tantalic oxide than is tartaric acid. The alkaline solution remains clear on acidification; in fact, it often is slightly turbid after the lixiviation, and, if so, a small excess of sulphuric acid removes the turbidity.

If from any cause a clear tartrate solution has not been obtained, the liquid is evaporated with a few c.c. of sulphuric acid till practically all the water is expelled. This may produce the desired effect; if not, a maximum concentration of sulphuric acid must be attained, which means destruction of the tartaric acid. That operation is not nearly so tedious as it is reputed to be. The tartrate liquor, containing about 5 c.c. of sulphuric acid, is evaporated on a hot plate till it blackens and foams. Strong nitric acid is then slowly dropped into the covered beaker from a tube inserted through the spout. The black colour is discharged, with copious evolution of red fumes. When this ceases, the beaker is uncovered and heated to the appearance of white fumes; if the liquid now remains colourless, all the organic matter has been destroyed; but if darkening again occurs, the treatment with nitric acid is to be repeated. During such treatment the sulphuric acid should not actually boil, but just fume. Finally, all the nitric acid is expelled in the usual manner. The cold residual acid mass, upon dilution with 20 per cent. tartaric acid, yields a clear solution.

Filter paper, tannin, oxalic and citric acids also are destroyed by the above procedure. Ammonium chloride is entirely removed by evaporation with a large excess of nitric acid; ammonium sulphate with *aqua regia*.

C. RECOVERY OF EARTH ACID FROM TARTRATE SOLUTION.—The subject has been very fully discussed under XVI and XVII (ANALYST, 1929, 54, 704, 709). It may be recalled that the bulk of earth acid is precipitated by hydrochloric acid added to the boiling tartrate solution ("tartaric hydrolysis") as *HP*, the balance

being recovered from the filtrate as the tannin precipitate *TP*. This mode of working has stood the test of numerous applications. One slight change has been made, which constitutes an improvement of practical importance. In Section XVII it was found necessary to re-treat all the weighed tannin precipitates and determine the impurities (chiefly  $\text{Fe}_2\text{O}_3$ ), the net weight of  $\text{M}_2\text{O}_5$  being obtained by difference. That procedure proved to have two disadvantages:

(1) The tannin precipitate is once more in solution, whereas it is required in the solid state for further treatment. (2) Any impurities that find their way into the solution of the tannin precipitate after it has been weighed, will cause a low result, since they will be deducted from the gross weight; as a matter of fact, the errors in the majority of test analyses under XVII are slightly negative. The following short description of the complete recovery procedure embodies the slightly modified purification method by which the drawbacks of the original process are obviated:

*Procedure.*—The boiling acid tartrate solution of the bisulphate melt (200 c.c.) is treated with 25 to 30 c.c. of strong hydrochloric acid, and the boiling continued for 10 minutes. The precipitate, *HP*, is collected, washed, ignited, and weighed. The combined filtrate and washings are nearly neutralised with fresh (silica-free) ammonia, boiled down to less than 150 c.c., and treated with ammonia, ammonium sulphide and acetate (5 grms.); the precipitated ferrous sulphide, if any, is filtered off after complete flocculation. The filtrate (200 c.c.) is slightly acidified with acetic acid, the hydrogen sulphide boiled off, and the boiling solution treated with a fresh solution of tannin (1 gm.). The precipitate, *TP*, is free from iron and silica; after ignition it only requires lixiviation (see *E* and *F*).

The distinctive feature of the modified procedure is, that the elimination of the impurities *precedes* the precipitation of *TP*.  $(\text{HP} + \text{TP}) = (\text{Ta}, \text{Nb})_2\text{O}_5$ .

*D. RECOVERY FROM OXALATE SOLUTION.*—The behaviour of the earth acids and certain mineral associates in tartrate solution differs from that in oxalate solution. (1) Precipitation of earth acid with strong mineral acid ("tartaric hydrolysis") is not feasible in oxalate solution; at least, the precipitation is very incomplete (XVI, *loc. cit.*).

(2) The tartrate solutions are not precipitated by ammonia; hence they can be freed from iron by precipitation with ammonium sulphide. This method is not applicable to oxalate solutions, from which ammonia precipitates the earth acids.

(3) Tannin precipitates titanium, zirconium, thorium, iron, aluminium, and uranium, together with the earth acids, from the neutralised tartrate solution (XVII, *loc. cit.*). From almost neutral oxalate solution half-saturated with ammonium chloride, tannin precipitates the earth acids and titanium, but not zirconium, thorium, aluminium, and iron (XVIII, ANALYST, 1930, 55, 612). Quantitative separations based on this differential precipitation are under investigation.

(4) The common accidental impurity co-precipitated by tannin from tartrate solution is iron, which is eliminated as has been described under *C*; in the case of

oxalate solutions, the common contaminant is calcium, the oxalate of which should be filtered off prior to the precipitation of the earth acids by tannin.

*Procedure.*—The distilled water, dilute ammonia, and strong ammonium chloride solution used should be lime-free, which is ensured by the addition of ammonium oxalate in the proportion of 1 grm. per litre and filtration after some days' standing.

The oxalate solution of the bisulphate melt is left to settle, and any calcium oxalate removed by filtration and washing with water. The clear solution is boiled, cautiously neutralised with *N* ammonia, treated with an equal volume of saturated ammonium chloride solution, and treated with tannin as described in Section XVIII for the precipitation of titania (*loc. cit.*, p. 608).

*E. FILTRATION AND WASHING OF PRECIPITATES.—Use of Filter Pulp.*—It should by now be almost unnecessary to recommend the addition of filter pulp in the filtration of amorphous and gelatinous precipitates. It is one of the most valuable expedients ever introduced into analytical practice. Apart from accelerating filtration and facilitating the washing, it imparts porosity to the ignited precipitate, and thus renders it easily soluble in fused bisulphate. Another advantage of filter pulp is its strong scouring action; after completing the transfer of earth-acid or tannin precipitates to the filter, we remove the last of the precipitate adhering to the glass by rubbing a little pulp round the inside of the beaker by means of a rubber-tipped glass rod. The beaker is thus easily and thoroughly cleaned.

*Hydrolysis Precipitate HP.*—The precipitate is collected on a No. 40 Whatman or No. 0 Swedish filter (11 cm.). The clear supernatant liquor may first be decanted into another beaker, filtered, and the beaker rinsed and discarded. This facilitates the incorporation of filter pulp with the precipitate in the original beaker; the mixture is then transferred to the filter. After draining, it is returned (if large) to the beaker, well stirred up with the wash-liquor, collected on the filter, and the washing completed. The wash-liquor is 2 per cent. ammonium chloride or hydrochloric acid. A slight opalescence sometimes seen in the filtrates from niobium precipitates is of no consequence, as the addition of ammonia prior to the iron precipitation has the effect of clearing the solution. The ferrous sulphide may be tested for  $M_2O_5$  by ignition and solution in hydrochloric acid; any slight white residue is added to *TP*.

*Tannin Precipitates* are very voluminous. That is their one disadvantage, in spite of which tannin has become an indispensable, and even the most important, reagent in earth-acid analysis. Fortunately, the ordinary facilities have proved adequate to overcome the difficulty. We apply suction filtration, using a platinum cone and No. 40 Whatman paper. The precipitates shrink considerably under suction; we use nothing larger than 12.5 cm. filters. In exceptional cases (*e.g.* when dealing with 0.25 grm. of titania), we divide the precipitate between two such papers. The precipitate should be returned to the beaker by a stream from the wash-bottle *before* it has become compressed and furrowed by the effect

of suction; it is thus removed without difficulty. It is churned up with the wash-liquor, and mixed with a little filter pulp, after which it is again transferred to the filter, and the washing completed. A 2 per cent. ammonium chloride solution is used for washing; in the case of precipitations from tartrate solution, it should contain a little tannin (about 0.5 gm. per litre).

For small tannin precipitates, filtration under atmospheric pressure is convenient; a loose-textured paper (No. 41 Whatman) is quite safe and ensures rapid filtration. The bulkiness of tannin precipitates increases in the order Ta-Nb-Ti-Al, *i.e.* with decrease in atomic weight.

**F. IGNITION AND PURIFICATION OF PRECIPITATES.**—A platinum crucible is as unnecessary for the ignition of the precipitates under discussion as it is undesirable for bisulphate fusions. When Weiss and Landecker assert that earth-acid and titania precipitates may be reduced by ignition in contact with filter paper to "blue-black" or "deep-black" oxides (*Z. anorg. Chem.*, 1909, 64, 80), we are forced to assume that their products were contaminated with undetected reducible impurities such as stannic or tungstic oxide. Whether they used platinum crucibles or not is not stated; at any rate, the permeability of platinum to reducing gases at high temperatures is an argument against its use.

Porcelain crucibles are very satisfactory, while the use of silica crucibles enables the operator to proceed at once with a bisulphate fusion of the weighed oxides. Having conducted some thousands of ignitions of earth-acid and titania precipitates in contact with filter paper in silica and porcelain crucibles, we can definitely state that we have never observed any perceptible discoloration to take place.

The time-honoured, tedious process of igniting hard, lumpy precipitates with addition of ammonium carbonate in a platinum crucible to constant weight is now out of date. The oxides obtained by ignition of precipitates, prepared as prescribed above, are soft, light powders which readily attain constant weight after application of the following simple process:

**Procedure.**—The moist precipitate (*HP* or *TP*) is heated in a tared porcelain or silica crucible on an asbestos mat until the paper is quite charred. The ignition is completed on a triangle; the crucible may be weighed as a check.

The ignited oxide is cautiously transferred to a 50 c.c. beaker, which is covered while a little 5 per cent. hydrochloric acid is added. If any oxide or filter ash adheres to the crucible, it is cleaned with the same acid, which is added to that in the beaker. After half-an-hour's hot digestion, the liquid is rendered slightly ammoniacal, the precipitate collected, washed with 2 per cent. ammonium nitrate, and ignited in the same crucible on an asbestos mat, then over a strong burner for 15 minutes. The crucible is weighed, and the net weight found by subtraction of the filter ash.

The lixiviation process removes small quantities of alkali and sulphur trioxide, the usual loss being of the order of 0.001 gm. The positive errors recorded in two of our earliest experiments on tartaric hydrolysis (*IX, ANALYST*, 1927, 52, 634,



Exps. 1 and 3) could only be due to incomplete washing uncorrected by leaching, which was adopted at a later date.

G. MICRO-TEST FOR EARTH ACIDS.—The tannin precipitates, on account of their bulky nature and characteristic colour, are invaluable in micro-work, all the more so as other earth-acid reactions are not sufficiently sensitive or specific for the purpose.

On many occasions we have searched the filtrates from *TP* for minute amounts of earth acids, so as to satisfy ourselves that the recovery was complete. After standing for a day or more, these solutions generally deposit a dark, flocculent alteration product of tannin, which is almost ash-free, and has not been found to contain earth acid.

Our procedure for the detection of earth acid in the tannin filtrates may serve as an example of micro-work. The liquor is evaporated with 100 c.c. of nitric, and 5 to 10 c.c. of sulphuric acid for the destruction of the ammonium salts, tannin, and tartaric (oxalic) acid. When colourless, the acid liquid is transferred to a silica dish in which the evaporation is pushed to complete dryness. The residue, consisting of the bisulphate originally used as a flux, is dissolved in hot water, the solution rendered slightly ammoniacal, and the small precipitate collected, washed, and ignited.

The constituents of the precipitate may be silica, earth acid, alumina, and ferric oxide; the possible presence of titania should always be taken into account in earth-acid work. The precipitate is treated in the usual manner with hydrofluoric acid and a drop of sulphuric acid in a small platinum cup made of foil; the residue is fused with a speck of bisulphate, and the mass dissolved in a c.c. or so of ammonium oxalate solution. The liquid—filtered, if turbid, through a tiny pad of filter pulp—is boiled in a 20 c.c. beaker and treated with a few mgrms. of tannin, an equal bulk of saturated ammonium chloride solution, and 0.2 *N* ammonia added, drop by drop, from the fine jet of a burette. Near the neutral point, the earth acids (and titania) will be precipitated as characteristic, yellow to red complexes, whilst iron and alumina remain in solution (*cf. D*). If a faint excess of ammonia is added, they will be precipitated, iron as a mauve, aluminium as a dirty-white complex, soluble on re-acidification. The earth-acid precipitate is collected on a 4 to 5 cm. filter, washed, ignited, and weighed; it is once more fused with bisulphate, dissolved in a little ammonium oxalate, and the liquid tested colorimetrically for titania with hydrogen peroxide and sulphuric acid.

Working in this manner, we have occasionally found a fraction of a mgrm. of earth acid in the liquors.

SUMMARY.—The following manipulations of fundamental importance in earth-acid analysis are discussed and described: bisulphate fusion, solution of the melt in oxalate or tartrate solution; tannin precipitation of the earth acids from tartrate and from oxalate solution; filtration, washing, ignition, and purification of precipitates; a micro-test for earth acids.

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## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### A COLOUR TEST FOR *o*-DIHYDROXY-PHENOLS.

IF to an aqueous solution of catechol or related *o*-dihydroxy-phenol, there are added acetic acid and ammonium molybdate, an intense reddish-brown colour is developed. The depth of the colour depends upon the concentration of the phenol.

The colour is doubtless associated with the formation of a molybdenum-polyphenol complex. Compounds of catechol and various metals have been described by Weinland and Sperl (*Z. anorg. Chem.*, 1925, **150**, 89), and Weinland and Maier (*Z. anorg. Chem.*, **150**, 217); Weinland, Babel, Gross and Mai (*Z. anorg. Chem.*, **150**, 177) have also shown that molybdic acid forms complex anions containing 1 and 2 molecules of pyrogallol or gallic acid. Martini has described a micro test for molybdenum by the use of catechol (*Anal. Assoc. Quím. Argentina*, 1926, **14**, 177; *Mikrochem.*, 1928, **6**, 63), and recently Paget (*Bull. Sci. Pharmacol.*, 1930, **37**, 537) has shown that adrenaline hydrochloride in 0.5 per cent. solution gives with ammonium molybdate solution a reddish-brown colour which changes to a greenish fluorescence after addition of sodium hydroxide.\*

The test, as I use it, seems to be fairly specific for the *o*-dihydroxy-phenols; it is carried out as follows: To 2 c.c. of an aqueous solution of the phenol are added 0.5 c.c. of glacial acetic acid and 1 c.c. of a concentrated (14 per cent.) solution of ammonium molybdate. There appears immediately a reddish-brown coloration which is stable.

The limits of sensitivity of the test are as follows:

Pyrocatechol	1 in 75,000
Pyrogallol	1 in 100,000
Protocatechuic acid	1 in 75,000
Protocatechuic aldehyde	1 in 75,000
Gallic acid	1 in 200,000
Adrenaline	1 in 60,000

The following phenols give little or no coloration: Guaiacol, resorcinol, quinol, phloroglucinol, orcinol, phenol. Salicylic acid gives a coloration in strong solution, the limit being 1 in 3000.

J. H. QUASTEL.

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\* Since this was written my attention has been drawn to the fact that Kedesdy (*Mitt. Kgl. Materialprüfungsamt*, 1907, **25**, 268) has used a nitric acid solution of ammonium molybdate for the colorimetric determination of gallotannin and gallic acid.

## THE pH VALUE OF CULTURE MEDIA.

IN describing "The Testing of Admiralty Disinfectant Fluid" in the February number of *THE ANALYST*, Messrs. Patterson and Frederick emphasise the importance of the pH value of the broth used in the test. They state that the method of preparing the broth in their laboratory is that of McIntosh and Smart (*Brit. J. Exp. Path.*, 1920, 1, No. 1, February).

The reaction specified for Admiralty broth is pH 7.6, but considerable variation from this figure may be obtained if McIntosh and Smart's method of control is used. It is our experience that a broth showing no coloration with phenolphthalein (and, therefore, conforming to McIntosh and Smart's technique) may have a reaction as high as pH 8.2.

The exact point at which indicator colour changes occur will always remain a matter of individual observation. McIntosh and Smart state that phenolphthalein first shows a pink colour at pH 7.9, but the generally accepted figure is pH 8.3.

In any case, a more rigid method of controlling the reaction of the broth seems desirable, and, as the use of comparators for pH control is now recognised to be quite satisfactory, it would appear to be much simpler and more accurate to specify a reaction of pH 7.6, using a reliable comparator with phenol red as indicator.

JAS. GIBSON.

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**Erratum:** In the Note by Messrs. Rideal and Sciver (April issue, p. 250, line 21):  
For "2 per cent. read "10 per cent."

## Notes from the Reports of Public Analysts.

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

## CITY OF BIRMINGHAM.

## REPORT OF THE CITY ANALYST FOR THE FOURTH QUARTER, 1930.

OF the 1127 samples examined, 1052 were informal, and 75 formal.

**NITRATES IN WATERED MILK.**—Nine samples of milk from one farm showed evidence of tampering, although only two contained less than 8.5 per cent. of solids-not-fat. In every case but one nitrates (derived from the water supply at the farm) were present in the milk. It was significant that from the time the

farmer knew that his milk was being sampled, there was a steady improvement in the quality of successive samples, but even in samples taken on the last day nitrates were still present in small amount, showing that water was still being added.

**TABLE VINEGAR.**—In two cases the bottle was definitely labelled "Table Vinegar," but the contents consisted entirely of artificial vinegar. In a case taken in Birmingham in 1926 (ANALYST, 1927, 52, 29) the magistrates decided that table vinegar should consist of malt vinegar. Both the vendors concerned were communicated with, and in both cases they agreed not to use the offending label in future.

**SAUSAGES.**—When a formal sample (containing 600 parts of sulphur dioxide per million) was bought, no label was given to the inspector, and there was no notice visible in the shop, but while the sample was being divided a card in the window, advertising some New Zealand mutton, was removed, showing the preservative notice underneath. Obviously, that could not be said to have been "conspicuously visible," and the vendor was prosecuted and fined £1.

**DYED MURCIA ORANGES.**—These were bought from a street barrow and were small oranges of a rich colour. When examined, they were found to be artificially coloured with a coal-tar dye. Similar oranges were bought in Birmingham some years ago (*cf.* ANALYST, 1925, 50, 183), and apparently the practice has been carried on for a considerable time. The Town Clerk's Department, however, considered that it would be inadvisable to prosecute as, in their opinion, it was impossible to prove by analysis that the sample did not consist of Murcia oranges. The fruit was of inferior quality, but, after dyeing, was an excellent imitation of a Tangerine orange. Incidentally, the fraud may be detected by examination of the calyx which, in the genuine orange, is green, and in the dyed article was coloured red.

**COFFEE EXTRACT.**—This was a proprietary article, and was shown, on analysis, to consist of dried aqueous extract of coffee. In the description on the label, however, a statement was made that the contents (weighing 1 ounce) were equivalent to more than half-a-pound of coffee. Coffee, however, yields about 25 per cent. of its weight of extract when treated with water, so that, at the most, 1 ounce would be equivalent to only four ounces of coffee, quite apart from the fact that the flavour and aroma of the original coffee are altered and partly lost. The firm was communicated with, and their explanation was to the effect that the average person does not extract everything out of ordinary coffee, and that 1 ounce of the extract will give as much usable beverage as is generally obtained from half-a-pound of ordinary coffee. This is obviously not what the ordinary purchaser would imagine the statement to mean, and the firm finally agreed to omit the sentence from the description both on the wrapper and on the tin.

**BEEF SUET.**—A sample contained 85 per cent. of fat, and the label stated that "8 oz. would go as far as 12 oz. of ordinary suet." This kind of statement is very misleading, and simply means that the housewife is careless enough to waste over one-third of every pound of raw suet she buys. Shredded suet certainly is prepared in a convenient form for use, but there is no need to exaggerate its qualities in this way. No satisfactory settlement has yet been made with the packers of this article.

H. H. BAGNALL.

## CITY OF SALFORD.

## ANNUAL REPORT OF THE CITY ANALYST FOR THE YEAR 1930.

Of the 3290 samples examined during the year, 1556 were bought under the Food and Drugs Act, and of these 50 (3·2 per cent.) were adulterated.

**CHEESE.**—The necessity for a Cheese Bill is shown by a number of Salford cases in past years, and the wide variation in the product is brought out by 20 samples purchased in April and May, the inspector in each case asking for "cheese." Arranged in order of fat content on the dry substance, the samples ranged from 69·0 per cent. to 33·8 per cent. of fat, while the prices varied from 1s. 4d. to 10d. per lb. While one may pay 1s. 4d. for a cheese containing only 23 per cent. of fat, it is yet possible to purchase one with 40 per cent. at 10d. per lb. Under present conditions all these cheeses must be classed as genuine.

**SULPHUR DIOXIDE IN DRIED APRICOTS.**—Two samples (formal and informal) from the same dealer contained 3000 and 2700 parts per million, respectively. During the past two years the average amount of sulphites found in dried apricots has been about 500 parts per million. There is no doubt that such excessive quantities of "preservative" are really the result of attempting to improve the appearance of an inferior article by bleaching. An interesting experiment was made to see how much sulphite remained after the fruit had been prepared for the table in the ordinary way (soaking overnight and boiling for 45 minutes with water and sugar). After such treatment 70 per cent. of the original amount still remained. On the instructions of the Health Committee, the London agents of the packers were communicated with. They agreed to withdraw all the consignment from sale, and to recall any portions of it that had already reached the hands of retailers.

**CASTOR OIL PILLS.**—These pills, though bought as "Castor Oil Pills," were actually labelled "Compound Castor Oil Pills," the word "compound" being in comparatively small type. They contained various vegetable laxatives, chiefly aloes, with, at most, 5 per cent. of castor oil. A deputation was received from the Wholesale Association, and finally it was agreed that the pills should be described as "Compound Laxative Pills," "Laxative Pills," "Compound Aperient Pills," or "Aperient Pills," to be followed in each case by the statement: "Each pill contains (the appropriate amount) of castor oil." It was further agreed that the description used should be in type at least as prominent as the words "Castor Oil" on the label and in the descriptive matter.

**STRENGTH OF SUNLIGHT.**—In general, the monthly figures showed that the active amount of sunlight received in the centre of the City is considerably less than the amounts received at the outlying stations.

*Influence of the Purity of the Potassium Iodide.*—During the year it has been discovered that the purity of the potassium iodide used affects the results obtained by the method previously described (ANALYST, 1927, 52, 641; 1929, 54, 101) to a considerable extent. During one week in July the determinations at the Regent Road Station were made in quadruplicate as follows:

Two qualities each of sulphuric acid and potassium iodide were used, the first being ordinary "pure" chemical reagents (which were of excellent quality), and the second being the finest quality obtainable from the manufacturers of fine

chemicals. From these, four test solutions were prepared each day and exposed, side by side, with the following results:—

		Iodine in a week. Mgrms.
1. Purest potassium iodide and purest acid	..	33·2
2. Ordinary potassium iodide and purest acid	..	39·7
3. Ordinary potassium iodide and ordinary acid	..	40·3
4. Purest potassium iodide and ordinary acid	..	34·6

In this experiment it will be seen that the greatest variation is caused by the quality of the potassium iodide, the results of Nos. 1 and 4 falling in one class, and those of Nos. 2 and 3 in another.

Chemical examination of the potassium iodide revealed practically no difference, each sample being of high quality. Titration of the "ordinary" sample pointed to the presence of a little potassium chloride (about 0·5 per cent.), though this was not indicated by qualitative test. The lesson to be learned is that only potassium iodide of the highest purity should be used in this test. There is no doubt, however, that the figures taken for a period represent a good comparative measure of the sunlight received at the different stations.

H. E. MONK.

## Department of Scientific and Industrial Research.

### FOOD INVESTIGATION. Special Report No. 40.

#### THE CORROSION OF THE TIN-PLATE CONTAINER BY FOOD PRODUCTS.\*

AFTER a review of work already done in connection with corrosion of tins by the contained foods, the apparatus used in connection with the experiments at the Low Temperature Research Station is described. The preliminary experiments indicate that the treatment of the steel may have a considerable effect on the rate of corrosion, and the value of the cold rolling process is emphasised. Further, the value of films of oxide is discussed, and the need for soundly constructed cans emphasised. The influence of the hydrogen-ion concentration on the corrosion of steel was studied in detail, and it was found that differences in the concentration at different points result in localisation of attack of the corroding medium, so that at high acidities exposed portions of the metal are particularly liable, and at low acidities the attack is directed towards pits, seams and other less exposed portions. Efficient exhaustion of the tins is specially important with low acidities. In the case of tin, elimination of air and other oxidising agents is very important, and corrosion of tin in the presence of the ferric salt is much more rapid than when free oxygen is present but iron absent. With a tin-iron couple the

\* Obtainable at H.M. Stationery Office, Adastral House, W.C.2. Price 1s. 6d. net.

corrosion of the iron is remarkably decreased by contact with the tin; contact with iron increases the rate of corrosion of tin; total corrosion of the couple is less than that of a similar area of iron; corrosion of the tin increases as the  $pH$  of the corroding medium decreases in the presence or absence of air, and any hydrogen liberated is always from the iron. Experiments with tin plate bore out these conclusions. A study of inhibitors and accelerators of corrosion showed that sugars, particularly beet sugar, colloids such as agar and gelatin, and tin salts are among the former, whilst sulphides are accelerators. The results of adding these substances are, however, not foregone conclusions, but involve several simultaneously operating factors. Discoloration of canned foods by tin occurs in the presence of anthocyanin pigments, and double lacquering of the tins is necessary in these cases for a first-class pack, or cool storage. Iron may cause blackening, by combination with tannin, and the conditions governing the formation of sulphides in various classes of food were investigated in detail, particularly for marine products, corn, meat, and fruit. The end part of the Report deals with practical experiments and considerations; the conditions found favourable for the formation of hydrogen swells are low acidity, lacquer on the inside of tin, presence of substances (e.g. sulphides) which accelerate corrosion with products of high acidity, storage at high temperatures, and inefficient cooling after processing, the presence of absorbers of tin salts, inefficient exhausting and insufficient headspace. Conditions favouring perforations are low acidity, lacquering, presence of oxygen (due to inefficient exhausting or minute pores in the tins), presence of anthocyanin pigments or other oxidising agents, presence of substances which render tin salts insoluble, and inefficient cooling and storage at high temperatures. It is recommended that attention should be paid to technical and mechanical details, including the adjustment of acidity in canning fruits by the addition of 0.3 to 0.5 per cent. of citric acid, care in selecting sugar free from sulphur compounds, and the use of beet sugar as an inhibitor of corrosion, or possibly the addition of agar. The appendix deals with the examination of canned fruits for factory control.

D. G. H.

## Standardisation of the Method of Presenting Results of the Analysis of Foods and Feeding Stuffs.

CONVENTION INTERNATIONALE POUR L'UNIFICATION DE LA  
PRÉSENTATION DES RESULTATS D'ANALYSE DES MATIÈRES  
DESTINÉES A L'ALIMENTATION DE L'HOMME ET DES ANIMAUX.\*

RESOLUTIONS for unification in the presentation of results of the analysis of food materials concern the expression of notation, quantities of material, volume, temperature, calorimetric determinations, pressure, specific mass, density, refractive index, polarimeter readings, acidity, alkalinity, reducing sugars, iodine and bromine values, and the factor for protein. In the case of brandies the special

\* *Ann. Falsificat.*, 1931, 24, 69-75.

regulations enact that esters should be expressed as ethyl acetate; aldehydes as acetaldehyde; higher alcohols as isobutyl or isoamyl alcohol, and volatile acids as acetic acid. They are to be expressed as mgrms. per litre, and as mgrms. per 100 c.c. of absolute alcohol contained in the brandy analysed; extractives and fixed acidity as grms. per litre, and the letters "C.I." indicate conformity with these expressions. The Convention is to be ratified at Paris as soon as possible, and the Governments concerned are to append their signatures up to July, 1931.

D. G. H.

## Ministry of Agriculture and Fisheries.

STATUTORY RULES AND ORDERS, 1931, No. 168.

### AGRICULTURAL PRODUCE (GRADING AND MARKING), ENGLAND.

THE AGRICULTURAL PRODUCE (GRADING AND MARKING) (CIDER) REGULATIONS, 1931, DATED MARCH 23, 1931, MADE BY THE MINISTER OF AGRICULTURE AND FISHERIES AS TO GRADE DESIGNATIONS AND GRADE DESIGNATION MARKS FOR CIDER.\*

In exercise of the powers conferred on him by the Agricultural Produce (Grading and Marking) Act, 1928, the Minister of Agriculture and Fisheries hereby makes the following regulations:—

1. Grade designations to indicate the quality of cider produced from apples and pears grown in England and Wales shall be as follows:—

SELECT CIDER (CHAMPAGNE PROCESS) or  
SELECT CYDER (CHAMPAGNE PROCESS)  
SELECT CIDER or SELECT CYDER

and the quality indicated by such grade designations shall be deemed to be as described in columns (2) and (3) of the First Schedule hereto.

2. A grade designation mark shall be any one of the grade designations specified in regulation 1 associated with the words "Empire Buying Begins at Home" and with the following mark, namely, a map of England and Wales in silhouette with the words "Produce of England and Wales" inscribed in a circle placed centrally in the map within which circle is a design representing the Union Jack, and which is more particularly described in the Second Schedule hereto.

3. These regulations shall come into operation on the 23rd March, 1931.

4. These regulations may be cited as the Agricultural Produce (Grading and Marking) (Cider) Regulations, 1931.

In Witness whereof the Official Seal of the Minister of Agriculture and Fisheries is hereunto affixed this twenty-third day of March, 1931.—CHARLES J. H. THOMAS.



## SCHEDULE I.

*Cider made from Apples and Pears grown in England and Wales.*

## GRADE DESIGNATIONS AND CHARACTERISTICS.

Grade designation. 1.	General characteristics. 2.	Special characteristics. 3.
SELECT CIDER (CHAMPAGNE PROCESS) or SELECT CYDER (CHAMPAGNE PROCESS).	Produced from clean and reasonably sound cider apples only, or from a suitable blend of clean and reasonably sound high acid and sweet and/or bitter sweet apples and/or of clean and reasonably sound pears only; no concentrated apple juice or other fruit juices to be used; no foreign acids to be permitted; no sweetening substances to be used other than pure beet or pure cane sugar; acetic acid must not be present in such quantities that it is discernible to the palate; and the total volatile acids of the acetic type present must not exceed 0.15 per cent., expressed as acetic acid; to be free from preservatives and/or artificial colouring agents prohibited by the Public Health (Preservatives, etc., in Food) Regulations in force for the time being; to be free from artificial bouquets and essences; to be free from disorders such as ropiness and sickness.	In the process of manufacture (1) the late stages of fermentation must take place in bottle, and (2) the deposit must be removed and the removal of deposit must be by the disgorging process after a suitable period of storing. Artificial carbonation is not permitted. To the pure, <i>i.e.</i> undiluted juice or battery diffusion juice of similar gravity, may be added not more than 25 per cent. of its own volume of a syrup made from pure cane or pure beet sugar (the syrup or its constituents may be added at any stage of manufacture). The original gravity of the finished product, whether pure juice cider or a cider to which syrup has been added, must not be less than 1.040 at 60° F.
SELECT CIDER or SELECT CYDER	Produced from clean and reasonably sound cider apples only, or from a suitable blend of clean and reasonably sound high acid and sweet and/or bitter sweet apples and/or of clean and reasonably sound pears only; no concentrated apple juice or other fruit juices to be used; no foreign acids to be permitted other than a <i>maximum</i> proportion of 1.0 gramme per litre (equivalent to 0.16 ounce per imperial gallon) of either tartaric or citric acid; no sweetening substances to be used other than pure beet or pure cane sugar; acetic acid must not be present in such quantities that it is discernible to the palate, and the total volatile acids of the acetic type present must not exceed 0.15 per cent. expressed as acetic acid; to be free from preservative and/or artificial colouring agents, prohibited by the Public Health (Preservatives, etc., in Food) Regulations, in force for the time being; to be free from artificial bouquets and essences; to be free from disorders such as ropiness and sickness.	To the pure, <i>i.e.</i> undiluted juice or battery diffusion juice of similar gravity, may be added not more than 25 per cent. of its own volume of a syrup made from pure cane or pure beet sugar (the syrup or its constituents may be added at any stage of manufacture). The original gravity of the finished product, whether pure juice cider or a cider to which syrup has been added, must not be less than 1.040 at 60° F.

SCHEDULE II. This gives the Grade Designation Mark.

**AGRICULTURAL PRODUCE (GRADING AND MARKING) ACT, 1928.**

**THE** following Circular (S.C. 12234) has been sent to the Local Authorities administering the Act:

SIR,—It has been brought to the notice of the Ministry of Agriculture and Fisheries that many of the Officers who have been instructed by Local Authorities to execute the above-mentioned Act are in some doubt as to the extent of their duties. The following observations are intended for their guidance:

The Act is primarily an amendment of the Civil Law, but it also contains certain criminal provisions which are of considerable importance. The Act may accordingly be considered under two headings—Civil Provisions and Criminal Provisions.

**CIVIL PROVISIONS.**—Under Sections 1 and 2, the Minister of Agriculture and Fisheries may make regulations prescribing grade designations for any kind of agricultural produce, and defining the quality indicated by such designations. Any person may use these designations, no special authority being required. The Minister has also prescribed a mark, generally known as the National Mark, which, when used in association with a grade designation, constitutes a grade designation mark. The Minister may authorise or empower a person or committee to authorise the application of grade designation marks, and persons thus authorised are known as "authorised packers."

Grade designations and definitions of quality and also grade designation marks are prescribed by regulations (Statutory Rules and Orders). A purchaser of produce to which a grade designation or National Mark is applied has a definite contract that the produce accords with the definition implied by the designation, and if it does not do so he has his remedy in respect of the breach of contract.

It may happen that where the definition includes a reference to the number, quantity, measure, gauge or weight of the goods, a seller, whether the original packer or subsequent handler who fails to fulfil the contract, may also fall under suspicion of having committed an offence against Section 2 (2) of the Merchandise Marks Act, 1887. As regards prosecutions under this last-mentioned Act, the Ministry is, under the Merchandise Marks (Prosecutions) Act, 1894, and the Regulations (S.R.O., No. 49 of 1914), made under that Act, the authority charged with this duty in all matters relating to agricultural and horticultural produce. Where, therefore, any suspicion of an offence against the Act of 1887 arises, the Ministry desires that the question of prosecution should be referred to it with a full report of the circumstances for decision as to the steps to be taken.

The use of grade designations and the National Mark is entirely voluntary, and the object of the Ministry is to encourage their use in order to secure the widest possible measure of standardisation of product, pack and package throughout the trade in home produce. For this reason, the Ministry wishes to dispel any idea that the adoption of the statutory grade designations and authorisation to apply the National Mark involve increased risk of incurring criminal liability. So far as the National Mark is concerned, it is believed that the machinery provided for its administration is sufficient to prevent the authorisation of fraudulent persons, or at least to ensure their expulsion from the select body of authorised packers before they have time to commit fraud in connection with the Mark. The use of the statutory grade designations without the concurrent use of the National Mark is, as yet, uncommon, and accordingly of no commercial significance.

Officers of Local Authorities are not, therefore, required to undertake any duties in connection with questions arising under Sections 1 and 2(1) and (2) of the Act.

**CRIMINAL PROVISIONS.**—It comes within the province of Local Authorities to deal with offences under Section 2 (3) (forgery or representation of the National Mark) and Section 2 (4) (the unauthorised application of the National Mark). Offences under Section 2 (3) are of a very serious character, and if they were committed it would be most desirable that the criminal should be brought to justice.

Local Authorities are also concerned with Sections 3 and 4 of the Act.

**Examination of Eggs.**—Section 3 requires that all eggs, whether of English, foreign, or Empire origin—that have been preserved in any way shall be marked before sale or exposure for sale. The manner in which such eggs must be marked is prescribed in the Agricultural Produce (Grading and Marking) (Eggs) Regulations, 1930. In the case of imported eggs which have been preserved, the marking is in addition to that prescribed in the Merchandise Marks (Imported Goods) No. 5 Order, 1928, as an indication of origin. Cold-stored and chemically-stored eggs

are exempted from this provision, and are dealt with under Section 4. At the moment, therefore, Section 3 applies only to eggs preserved in lime water, water-glass, or oil.

It can usually be proved by analysis when these methods of preservation have been employed, but as it is always possible that individual eggs may fail to afford the proof required, it may be necessary to take a series of samples in cases where there is reason to suspect that such eggs are being sold unmarked.

In a prosecution under Section 3 of the Act, it is not necessary to prove the method of preservation. It is sufficient to show that the eggs have been subjected to a process of preservation other than cold storage or chemical storage.

The Government Chemist has been investigating tests by which the fact that eggs have been preserved in oil, water-glass or lime may in certain cases be detected, and the Ministry understands that, in any case, where a Public Analyst desires information as to these methods in connection with samples which have been submitted to him under the Act, the Government Chemist will be glad to communicate to him the latest results. Communications on this subject should be addressed to the Secretary, Ministry of Agriculture and Fisheries.

Section 4 requires that all premises used by way of trade or for purposes of gain for the cold storage or chemical storage of eggs, of whatever origin, must be registered, and that all *British* eggs which have been kept in cold storage or chemical storage must be marked before being removed from the storage premises. The manner of marking is prescribed in the Agricultural Produce (Grading and Marking) (Eggs) Regulations, 1930.

Section 4 also makes it an offence for any person to remove, alter, or obliterate any mark which was borne by an egg at the time it was stored in cold storage or chemical storage premises, or for anybody to sell or expose for sale any eggs from which such a mark has been removed or on which the mark has been altered or obliterated.

As a result of the foregoing provisions, the only *unmarked* eggs that may be lawfully offered for sale in the home market are fresh eggs (*i.e.* eggs not preserved, cold-stored, or chemically stored) produced in Great Britain and Northern Ireland.

The Ministry has its own inspection service in connection with the grading of produce and the administration of the National Mark Schemes, and should the Ministry's Inspectors, in the course of their duties, obtain any information as to contraventions of Section 2 (3), Section 2 (4), Section 3, or Section 4 of the Act, they will communicate the evidence to the appropriate officer of the Local Authority, with a view to the taking of such further action as the Local Authority may think fit.

I am, Sir, your obedient servant,

(Signed) CHARLES J. H. THOMAS.

March 31st, 1931.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Determination of Lactic Acid in Milk and Milk Products.** L. H. Lampitt and M. Bogod. (*Compt. rend. du Neuvième Congrès de Chimie Industrielle; Chim. et Ind.*, Special No., 510-5 (March, 1930).)—The method depends upon the oxidation of lactic acid to acetaldehyde by means of potassium permanganate, as suggested by Boas (*Deutsch. Med. Wochenschr.*, 1893, 19, 340) and later developed by von Furth and Charnass (*Biochem. Z.*, 1910, 26, 199) and Embden (*Abderhalden's Handbuch der biochemischen Arbeitsmethoden*, 1912, 5, 12, 55). For the determination the following reagents are required:—Sodium hydroxide, 0.1 N; sulphuric acid, 0.1 N; phosphotungstic acid reagent (Hinton and Macara, *ANALYST*, 1927, 52, 677); saturated solution of copper sulphate; pure

powdered calcium hydroxide; sulphuric acid, 5 *N*; potassium permanganate, 0.004 *N*; standardised (0.1 *N*) iodine solution; sodium bisulphite, approx. 0.02 *N*.

*Procedure.*—To 10 grms. of milk, 4 grms. of condensed milk or 1 gm. of dried milk are added 10 ml. of 0.1 *N* sodium hydroxide and about 30 ml. of water. The mixture is stirred, heated to boiling, and the liquid transferred to a 100 ml. measuring flask and cooled, and to it are added 10 ml. of 0.1 *N* sulphuric acid. This is followed by 5 ml. of phosphotungstic acid, and water is added to the mark; the mixture is well shaken and filtered, the first 10 ml. of filtrate being rejected. Twenty ml. of the filtrate are accurately measured into a 50 ml. flask, and 2 ml. of saturated copper sulphate added, followed by 3 grms. of calcium hydroxide. The whole is mixed, and water is added to the mark; the flask is shaken and allowed to stand for 15 minutes, with occasional shaking, and the contents are transferred to a centrifuge tube. After centrifuging for 5 minutes at 2000 to 2500 R.P.M. the supernatant liquid is filtered through a fine filter paper. Twenty-five ml. of this filtrate are accurately measured into a 250 ml. distillation flask which contains a little talc and 10 ml. of water; 1 ml. of 5 *N* sulphuric acid is added, and the flask is attached to a condenser; a dropping funnel is attached, and 100 ml. of 0.004 *N* potassium permanganate are poured into the funnel. The adaptor at the end of the condenser is made to fit into a flask of 100 to 110 ml. capacity, containing 10 ml. of sodium bisulphite solution; the end of the adaptor dips below the level of the bisulphite solution. A narrow tube from the flask extends to a U-tube containing 1.0 ml. of 0.01 *N* iodine solution to which 10 ml. of cold water have been added; both the receiving flask and the U-tube are immersed in ice. The distillation flask is now heated with the tip of a Bunsen flame just touching the bottom of the flask; when boiling begins, the permanganate solution is allowed to run, drop by drop, into the boiling liquid. The dropping is so regulated that 100 ml. may be added in 50 to 60 minutes. In this period, from 80 to 100 ml. of liquid are distilled. The receiving flask is now removed, the distillate and contents of the U-tube are rinsed into a titration flask, and the excess of bisulphite titrated with standard 0.01 *N* iodine solution; allowance is made for the iodine in the U-tube. Ten ml. of the bisulphite solution are titrated at about the same time. The difference between the titrations, multiplied by 0.45, gives the amount of lactic acid in mgrms. Determinations on known quantities of lactic acid added to milk powder showed a loss of acid due to the clarification of approximately 10 per cent.; a more accurate result for the lactic acid present is, therefore, obtained by multiplying the amount found by 10/9. Determinations have been carried out on milk, condensed milk and dried milk of different degrees of acidity, with and without added alkali. It has been shown that the total lactic acid content of normal milk is less than the equivalent of the acidity determined by titration, and that this difference diminishes or changes in sign if added alkali is present.

**The Fat of Sow's Milk.** O. Laxa. (*Ann. Falsificat.*, 1931, 24, 87–88.)—Sow's milk was dried with sand, the residue extracted with ether, and the fat dried in a current of carbon dioxide. The granular, clear, brown-yellow fat had

the following characteristics:—Solidif. pt., 17–18.5° C.; m.pt., 28° C.; butyro refractometer reading at 40° C., 52; saponification value, 193.9; iodine value, 58.2; Hehner value, 93.7; Reichert–Meissl value, 2.1; Polenske value, 1.2; solidif. pt. of insoluble acids, 36.5–37.5° C.; m.pt., 39–40° C.; iodine value, 61.9; molecular weight, 276.9. The melting point of the saturated acids was 60.5, and their molecular weight 256, and the composition of the fat is deduced as oleic acid, 64.5; palmitic, 26.6; myristic, 2.6; volatile acids (caprylic and capric), 1.4; and glycerol, 4.9 per cent.

D. G. H.

**Determination of Starch in Flour by Diastase and Acid Hydrolysis.**

**B. G. Hartmann and F. Hillig.** (*J. Assoc. Off. Agr. Chem.*, 1931, 14, 112–116.)—The results of further work on peptic digestion of starch-containing materials prior to diastatic conversion of the starch (*ANALYST*, 1927, 52, 160) show that the yield of starch is materially increased by this procedure. In the peptic digestion the acidity is adjusted to correspond with 5 c.c. of *N* hydrochloric acid per 100 c.c. of substrate. After this digestion the mixture is carefully neutralised with alkali, then slightly acidified with hydrochloric acid, and, finally, treated with calcium carbonate to ensure the virtual neutrality required for the proper function of the diastase. It is shown that the action of the pepsin consists solely of digestion of the proteins, the starch being thereby rendered accessible to diastatic conversion. In addition to this modification of the official method (*Assoc. Off. Agr. Chem., Methods of Analysis*, 1925, 119), the preliminary treatment of flour, etc., with ether and alcohol to free it from sugars and fat is simplified and rendered more reliable. Full details of the procedure are given. The percentages of starch found in this way in four commercial starches and in wheaten flour are slightly lower than those obtained by subtracting from 100 the sum of the percentages of moisture, fat, protein ( $N \times 5.7$ ) and ash; the undetermined matter, probably crude fibre, etc., amounted to 0.5 per cent. with the wheaten flour, and varied from 0.1 to 0.9 per cent. with the commercial starches examined.

T. H. P.

**Increase in the pH Value of White and Yolk of Hens' Eggs.** **P. F. Sharp and C. K. Powell.** (*Ind. Eng. Chem.*, 1931, 23, 196–199.)—The pH value of the white of untreated eggs rises very rapidly after the eggs are laid owing to the escape of carbon dioxide, and increases more rapidly as the temperature rises. For instance, from an initial value of 7.60, a pH value of 9.25 will be reached in two days at 37° C., in five days at 16° C., and in ten days at 2° C. After long periods of storage, particularly at the higher temperatures, the value begins to decrease. In the case of the yolks, the pH value increases at a slower rate. Dilution of the white and yolk has but little effect on the increase of the pH value.

W. P. S.

**Determination of Small Proportions of Invert Sugar in Raw Sugars.**

**L. Eynon and J. H. Lane.** (*J. Soc. Chem. Ind.*, 1931, 50, 85–86T.)—In the determination of invert sugar in unclarified solutions of sugar products (*ANALYST*, 1923, 48, 277; 1924, 49, 90), the errors due to reducing non-sugars and to calcium salts act in opposite senses. With low-grade products, such as molasses, the nett

effect of these two errors may be serious, but with raw sugars such effect is negligible. The invert sugar Table I (ANALYST, 1923, 48, 221) does not make provision for sugar samples containing less than 0.3 per cent. of invert sugar, but these cases are met by dissolving the material (25 grms.) with such quantity of neutralised invert sugar solution that the whole, after being made up to 100 c.c., contains 0.1 per cent. of added invert sugar. The titration is then carried out as accurately as possible, the percentage of invert sugar in the sample being obtained by deducting 0.1 from the percentage found and multiplying the remainder by 4. This procedure renders possible the determination of 0.01 per cent. or even less of invert sugar, and is useful also for testing the purity of sucrose preparations intended for purposes of standardisation. The acid, standard 1 per cent. invert sugar solution, prepared as already described (*J. Soc. Chem. Ind.*, 1923, 42, 34T), is remarkably stable, its titre remaining practically unchanged for many months, despite the formation of a small quantity of mould mycelium.

The following table, referring to 10 c.c. of Fehling solution, gives the burette readings for solutions containing 25 grms. of sugar sample, plus 0.1 grm. of added invert sugar per 100 c.c. It applies strictly to solutions containing 25 grms. of sucrose per 100 c.c., but the less amount given by 25 grms. of raw sugar introduces an error negligible in ordinary work.

Volume of solution required. c.c.	Invert sugar in sugar sample. Per Cent.	Volume of solution required. c.c.	Invert sugar in sugar sample. Per Cent.	Volume of solution required. c.c.	Invert sugar in sugar sample. Per Cent.
20	0.464	28	0.209	36	0.066
21	0.423	29	0.188	37	0.052
22	0.384	30	0.168	38	0.040
23	0.348	31	0.148	39	0.028
24	0.315	32	0.128	40	0.017
25	0.284	33	0.111	41	0.008
26	0.257	34	0.095	41.7	0.000
27	0.232	35	0.080		

T. H. P.

**Isolation of Quinic Acid from Fruits.** E. F. Kohman and H. H. Sanborn. (*Ind. Eng. Chem.*, 1931, 23, 126.)—Quinic acid may be isolated from prunes and cranberries by a process depending on the solubility of the lead and calcium salts of the acid, compared with the relative insolubility of the same salts of other inorganic acids. Although the separation is not strictly quantitative, there are indications that prunes and cranberries contain about 1 per cent. of quinic acid; the acid is also present in grapes.

W. P. S.

**Determination of Rancidity in Oils and Fats.** A. Taffel and C. Revis. (*J. Soc. Chem. Ind.*, 1931, 50, 87–91T.)—In order to eliminate the disturbing effect of the presence of peroxides in the ethyl ether used in the Kreis test for rancidity, and also the emulsification due to ether, a few drops of a 5 per cent. solution of phloroglucinol in alcohol are used in place of the 10 c.c. of 0.1 per cent. ethereal solution of the original test. Ten c.c. of oil (or 5 c.c. of a very rancid oil) are shaken

with 10 c.c. of concentrated hydrochloric acid for 30 seconds, 10 drops of 5 per cent. alcoholic phloroglucinol then added, the mixture shaken for 30 seconds, and set aside for 1 or 2 minutes, and the colour noted. The test, as thus used, was found more satisfactory, and in some cases more delicate than the usual Kreis test. Oils which have become rancid or have been air blown at fairly moderate temperatures contain (a) certain peroxides easily reducible by hydriodic acid, and those which have become rancid or been blown at higher temperatures such as 120° C. contain, in addition to a large proportion of these substances, other peroxides (b) which offer more resistance to reduction by hydriodic acid. The amount of each type of oxidation product may be determined, and the method depends on the fact that the amount of iodine set free by a rancid oil in the presence of barium iodide may be considered a measure of the peroxides or reducible oxygen in the oil. (a) Oils with a slight degree of rancidity, as shown by the Kreis test. Ten grms. of the oil are added to 40 c.c. of glacial acetic acid, followed by 2 grms. of anhydrous barium iodide (or 2 c.c. of 50 per cent. potassium iodide solution). After shaking, the mixture is poured into 100 c.c. of water, the bottle rinsed with 20 c.c. of water, and the liquid titrated with 0.1 N thiosulphate solution in the presence of starch. A blank is carried out without oil, and the titration gives a measure of the easily reducible peroxides. If the oil is strongly rancid the same ingredients are put in a 60 c.c. bottle, the remaining air displaced with carbon dioxide, and the stoppered bottle left in boiling water for 2 minutes, after which it is shaken, put back for 2 minutes, and again shaken, the hot contents poured into 150 c.c. of water, and the titration carried out as before. (b) The proportions of oil, barium iodide and acetic acid necessary to give the maximum thiosulphate titration must be found by experiment, but 1.25 to 2.5 grms. of oil, 10 to 20 grms. of barium iodide, and 100 c.c. of acetic acid are usually suitable. The mixed reagents are placed in a 150 c.c. distilling flask, air is displaced with carbon dioxide, and the whole heated to the b.pt. and kept just boiling for 30 minutes in a current of carbon dioxide. The flask is cooled, the contents poured into 400 c.c. of water containing starch solution and dilute sulphuric acid, and titrated, the titration giving the measure of all the difficultly reducible oxides. These methods were tested on B.P. liquid paraffin containing benzoyl peroxide, and satisfactory agreement with the calculated figures was obtained. The procedure has been worked out in detail for arachis oil, but the methods are apparently of universal application to all types of rancidity, and give definite and reproducible figures distinguishing between the two types of rancidity.

D. G. H.

**Oil from the Seeds of *Ribes rubrum* L. (Red Currant).** A. Jermstad. (*J. Pharm. Chim.*, 1931, 123, 243-244.)—The hard dried seeds of the red currant cultivated in Norway, yielded to ether 20.4 per cent. of a yellow oil of bland taste, and having the following characteristics: Sp. gr. at 20° C., 0.9311;  $n_D^{20}$ , 1.4801; acid value, 3.1; saponification value, 193.3; and iodine value, 176.3. These figures agree with those for the oil obtained from fruit grown in other countries.

D. G. H

**Solubility of Tea-seed Oil in Alcohol of Varying Concentrations.**

**K. Hashi.** (*J. Soc. Chem. Ind., Japan*, 1931, 34, 64B.)—A purified and decolorised sample of tea-seed oil having the following properties was used: Sp. gr. at 25°/25° C., 0.9125;  $n_D^{25}$ , 1.4650; acid value, 0.53; iodine value (Wijs), 77.7. The solubility of the oil in alcohol and water mixture was measured by Alexejeff's method. Known quantities of oil and solvent were placed in a closed glass tube, heated with shaking, and the temperature noted at which both phases merged into one. The clouding point on slow cooling with shaking was also determined. The results are expressed in the following table:

Alcohol per cent., by weight.	Critical temp. °C.	Critical concentration (oil per cent.).
99.75	71.0	40.8
95.50	99.8	41.5
90.45	129.8	44.0
85.50	155.0	48.0

The results are also given in graph form.

R. F. I.

**Solubility of Rape Oil in Alcohol of Varying Concentrations.**

**K. Hashi.** (*J. Soc. Chem. Ind., Japan*, 1931, 34, 66B.)—Purified and decolorised rape oil was used, having the following constants: Sp. gr. at 25°/25° C., 0.9117;  $n_D^{25}$ , 1.4690; acid value, 0.09; iodine value (Wijs), 99.5. The same technique was employed as in the preceding abstract. The results are given in tabular and graphical form:

Alcohol per cent., by weight.	Critical temp. °C.	Critical concentration.
99.75	86.3	38.5
95.50	113.0	40.3
90.45	140.3	43.0

R. F. I.

**Use of Acridine Dyestuffs for the Determination of Nitrites.**

**W. M. Rubel.** (*Z. Unters. Lebensm.*, 1930, 60, 588–592.)—"Rivanol" (Koremann), 2-ethoxy-6,9-diamino-acridine hydrochloride,  $C_2H_5O.C_8H_7CN(NH_2).C_8H_7NH_2.HCl$ ,  $3H_2O$  is a yellow-green antiseptic, which has a solubility of 1:260 and is fairly stable in the dark. If 0.5 c.c. of a 0.1 per cent. solution and 0.5 c.c. of hydrochloric acid (sp. gr. 1.06) are added to 10 c.c. of a 2- to 100-fold dilution of the liquid to be investigated, a yellow-green to orange or red colour is obtained, depending on the amount of nitrite present. This may be matched against a scale of standard solutions containing (e.g.) 0.1 (yellow) to 0.001 mgrm. (red) of  $N_2O_3$  (as sodium nitrite) in 10 c.c., and treated in the same way, the final comparison being made in a colorimeter of Duboscq type. The colour, which results from diazotisation of the amino-group, changes to yellow on heating, and is stable in the light for 1 hour, when a brown precipitate appears, but for longer in the dark. Its sensitiveness (0.001 mgrm./10 c.c.) is greater than that of the *m*-phenylenediamine reaction, and equal to that of the Griess reaction, but the colour is stabler than that of the latter and, unlike it, is unaffected by phenol and



ammonium salts, and is only slightly suppressed by thymol. Amino-acids, less than 10 per cent. of sodium chloride, nitrates, glucose and lactic acid have no effect, aldehydes weaken the colours, and free iodine produces a green-blue colour. Free ammonia, which interferes with the colour by alteration of the pH value, must be neutralised. Satisfactory results were obtained with sugars and extracts from meats. J. G.

**New Lead Iodide Double Salt of Trigonelline.** K. Lendrich and F. Mayer. (*Z. Unters. Lebensm.*, 1930, **60**, 569-575.)—Raw, whole coffee beans were boiled beneath a reflux condenser with 70 per cent. alcohol, caffetannic acid separated, and caffeine (simultaneously extracted with the trigonelline) was removed from the alcoholic extract by means of chloroform, the alcohol then expelled by evaporation under reduced pressure, and the aqueous solution cleared with lead acetate and treated with solutions of bismuth and potassium iodides. A suspension in water of the resulting brick-red precipitate was treated with hydrogen sulphide and a slight excess of hydriodic acid, and lead acetate added to the filtered liquid, which was then again filtered immediately to remove the bulk of the free lead iodide. Trigonelline lead iodide was deposited, on standing over-night, in dense golden needles,  $C_7H_7NO_3 \cdot PbI_2$  (m.pt.  $217^\circ C.$ , with decomposition), and was freed from lead iodide by recrystallisation from warm water, and dried at  $105^\circ C.$  This salt was also prepared from the free base by addition of a mixture of lead acetate and iodide solutions containing  $1/3$  of the calculated equivalent quantity of the latter. The base itself was prepared from the coffee-extract by separation as the mercuric chloride salt; the chloride was then produced by the action of hydrogen sulphide and treated with silver oxide. J. G.

**Assay of Pyramidon by the Silver Cyanide Method.** R. Machtou. (*J. Pharm. Chim.*, 1931, **123**, 329-333.)—One grm. of the pyramidon is dissolved in water and the solution made up to 100 c.c. To 50 c.c. of this solution are added 50 c.c. of a 5 per cent. solution of mercuric chloride, and the solution is vigorously shaken and filtered. The mercury in 10 c.c. of filtrate is determined by the silver cyanide method, using a solution made by pouring 0.05 *N* silver nitrate solution into 100 c.c. of water, to which have been added 10 c.c. of ammonium hydroxide and 1 c.c. of 10 per cent. potassium iodide solution, adding this until a distinct opalescence persists. If "*n*" is the quantity of 0.05 *N* silver solution used,  $n/2$  subtracted from 10 (*a*) (the amount of silver solution corresponding with the mercury in the solution) is the quantity equivalent to the cyanogen combined with the mercury. This value may be greater or less than 5.5. In the latter case the Denigès formula  $A = a \times 0.192$  is used. In the former case the mercuric filtrate is diluted to twice the volume, and the factor multiplied by 2. In order to determine the amount of mercury fixed by the pyramidon, the percentage of mercury in the 50 c.c. of the solution used for precipitating the pyramidon must be known; it is determined on 10 c.c. of the solution made up to 50 c.c., 10 c.c. of this being used. The method breaks down if the pyramidon is adulterated with antipyrin but was otherwise found very accurate for varying quantities of pyramidon. D. G. H.

**Direct Determination of Available Carbon Dioxide in Baking Powder.**

**M. R. Coe.** (*J. Assoc. Off. Agr. Chem.*, 1931, 14, 99–102.)—The two separate determinations required by the gasometric method for ascertaining the available carbon dioxide in baking powder (*ibid.*, 1923, 6, 453; *A.O.A.C., Methods of Analysis*, 1925, 305) may be reduced to a single determination by use of the following procedure: 25 c.c. of 5 per cent. ammonium sulphate solution are run into the 250 c.c. decomposition flask containing 1.7 grm. of the baking powder, the liquid being then kept at the boiling point until evolution of gas ceases and afterwards cooled to room temperature. When equilibrium is established, usually in about 15 minutes, the volume of the available carbon dioxide is read on the graduated tube and suitably corrected for temperature and pressure. If the total carbon dioxide in the sample is also required, this may be determined on the same portion of material without detaching the flask from the apparatus: The burette containing a few c.c. of the ammonium sulphate solution is filled with dilute sulphuric acid (1+5), 25 c.c. of which is run into the flask. The liquid is heated almost to boiling point and allowed to cool as before. The volume of gas then read is diminished by the volume of acid used; the remainder, corrected for temperature and pressure, represents the total carbon dioxide. The use of ammonium sulphate solution in this process ensures satisfactory evolution of carbon dioxide from baking powders of all varieties, acts as a protein-coagulant with powders containing egg-albumin, and thus destroys foam, and obviates the low results given by phosphatic or egg-albumin powders when distilled water is employed. The details of the operations are described.

T. H. P.

## Biochemical.

**Studies on Arginine. I. Rate of Catabolism of Arginine in Rats, including Method for Determination of Arginine in Biological Material.**

**V. C. Klech, J. M. Luck and A. E. Smith.** (*J. Biol. Chem.*, 1931, 90, 677–696.)—A volumetric method is described in detail for the determination of arginine in protein-free tissue extracts. The arginine is converted in the presence of arginase to ornithine and urea, and the latter is determined as dixanthidryl urea by oxidation with potassium dichromate and sulphuric acid. An alternative and more rapid method was devised, the principle of which was heat coagulation for removal of the tissue proteins and the use of urease for destruction of the pre-formed urea, and this was compared with the first (tungstic acid) method. However, the arginine values obtained by the heat coagulation method were too low, and added arginine was unsatisfactorily recovered, but with the tungstic acid method the extra arginine was recovered fairly well. In fasting female rats the arginine content was found to average 26.1 mgrms. per 100 grms. of muscle, and 27.5 mgrms. per 100 grms. of liver-free carcass. The values for liver were omitted because of fluctuations, probably due to the great activity of the arginase in the macerated organ, which gave to the liver values an uncertain significance. An arginine content of 25 or 30 mgrms. per 100 grms. of tissue corresponds with 2 or 2.5 mgrms.

of arginine  $\alpha$ -amino nitrogen. This in turn represents 4 or 5 per cent. of the non-protein amino nitrogen content of rat muscle, liver, or carcase. On the assumption that all of the non-protein amino nitrogen is present in the form of amino acids with a mean molecular weight of 120, the content of the latter would be about 390 mgrms. per 100 grms. of tissue. Thus arginine would account for about 6 or 7 per cent. of the non-protein amino acids of rat muscle and carcase. These analyses demonstrate clearly the existence of arginine as a constituent of vertebrate muscle. This is not in harmony with the conclusions of Kutscher and Ackermann (*Z. Biol.*, 1926, **84**, 181), who, in summarising their extensive investigations on the extractions of muscle expressed the generalisation that arginine, though a characteristic constituent of invertebrate muscle, was absent from the vertebrates. Arginine, administered to fasting rats by subcutaneous injection, was rapidly catabolised. Less than 12 per cent. of the injected nitrogen was present at any time in substances other than urea and arginine. Ornithine, which presumably would be formed, must have been catabolised almost as rapidly as arginine. It is proposed to correlate these and related observations on the metabolism of amino acids with data on oxygen consumption and carbon dioxide production.

P. H. P.

**Effect of Heat upon the Biological Value of Cereal Proteins and Casein.**

**A. F. Morgan.** (*J. Biol. Chem.*, 1931, **90**, 771-792.)—A brief summary of recent literature on studies on the heat denaturation of proteins is given, and the experiments of the author are described in detail. Preliminary growth experiments with young rats showed that the protein of cereals subjected to dry heat or toasting at approximately 200° C. for 45 minutes or to similar procedures during manufacture is not well utilised for growth. Cooking with water had but little similar effect, and caramel formation during toasting was found to account for only a small part of the injury. The addition of 5 per cent. of casein to these toasted diets very nearly made up the discrepancy between the latter and correspondingly supplemented raw diets, thus indicating that the deficiency observed lay in the protein fraction of the toasted diet. When young rats were given diets which contained raw and toasted (heated at 150° C. for 30 minutes) wheat gluten as source of protein at 9, 12, 15, 18, 21 and 24 per cent. levels (crude protein), in a diet otherwise adequate, the growth in 56 days per gm. of protein eaten was found to be 1.29 to 1.40 for raw gluten at all levels up to 18 per cent., and to fall to 1.15 and 0.98 at 21 and 24 per cent. The corresponding figures for the toasted gluten were 0.94 to 1.09, 1.12 and 0.86. The maximum growth rate on both raw and toasted diets was seen at the 18 per cent. level, but the toasted gluten supported less growth than the raw, both absolutely and relatively to the amount eaten at all levels. Male and female animals were used in about equal numbers in all groups, and the factors were averaged, since differences in the rates of growth per gm. of protein eaten for the two sexes appeared to be insignificant. The biological values of raw, water-cooked and toasted whole wheat protein, alone and supplemented by 5 per cent. of casein, as determined by nitrogen balance on young rats

according to the modified Mitchell method, were found to be 64, 67 and 52 for wheat alone, and 67, 75 and 69 when supplemented by the casein, and in a similar comparison of biological values of raw and toasted wheat gluten at an approximately 12 per cent. level the figures obtained were 66 and 54. Large mature rats used for a similar study of raw and toasted wheat gluten gave values of 83 and 64; another test with raw and toasted casein at an 8 per cent. level gave values 66 and 53. In all cases the differences between the biological values of raw and toasted proteins were found to be more than 6 times as great as their probable errors. It is emphasised that the *digestibility of the toasted proteins was but little different from that of the raw*, particularly in the older animals, and the unexplainable loss of nitrogen occurred chiefly in the urine; thus the change produced by the heat treatment lies probably in the assortment or availability of the amino acids absorbed.

P. H. P.

**Effect of Drying and Sulphuring on Vitamin C Content of Prunes and Apricots.** A. F. Morgan, A. Field and P. F. Nicholls. (*J. Agric. Res.*, 1931, 42, 35-45.)—In a study of the antiscorbutic property of peaches by Morgan and Field (*J. Biol. Chem.*, 1929, 82, 579; ANALYST, 1929, 54, 483) all of the fruit was from the same orchard, and the fresh fruit was tested along with the sun-dried and dehydrated products, sulphured and unsulphured. The sulphured peaches, both sun-dried and dehydrated, were found to have retained the full antiscorbutic value of the fresh fruit, but the unsulphured apparently retained none. Similar tests with prunes and apricots, involving a larger variety of drying conditions, were also reported to have been made, but were not then described in detail. The questions were raised: (1) Does sulphur dioxide protect other fruits equally well? (2) If so, how much is required for such protection? (3) How is the protective effect achieved? Each of these questions is now answered, in part at least. The vitamin C content of frozen fresh prunes and apricots and of prunes and apricots dried by various methods has been determined by biological technique; this is described. The fruit was prepared as described by Morgan and Field (*J. Biol. Chem.*, 1929, 82, 579; ANALYST, 1929, 54, 483; and *J. Biol. Chem.*, 1930, 88, 9; ANALYST, 1930, 55, 643). Doses of the different fruit products were given to guinea pigs for 60 and 90 days, and the rates of growth of the animals receiving the various doses were compared. Examinations were also made for symptoms of scurvy, and in cases where animals died because of insufficient protection from scurvy the length of the survival period was determined. The 60-day period is shown to be as effective for assay of vitamin C as the 90-day period. Frozen fresh prunes of two crops retained the vitamin C satisfactorily, but frozen fresh apricots packed in cases which were not evacuated, lost all of this property. A second lot packed in cases which were evacuated and filled with nitrogen before the fruit was frozen, retained the vitamin. The difference is ascribed to retention of tissue respiratory oxygen in the unevacuated lot. Sulphured, dehydrated and sun-dried prune products retained the vitamin C of the fresh fruit satisfactorily only when the fruit was dipped in lye in the usual commercial fashion before the sulphur dioxide treatment. This is ascribed to better penetration by the protecting

sulphur dioxide after the lye dipping. All unsulphured products of both fruits, whether sun-dried or dehydrated, were without antiscorbutic value. The dehydrated products, both prune and apricot, retained the vitamin C more completely than did the corresponding sun-dried fruit. The dehydrated and sun-dried apricots, containing 450 to 500 or more parts of sulphur dioxide per million, retained the antiscorbutic property more or less completely. With less than this amount, all products lost this property completely. The tendency to loss of vitamin C in frozen, sun-dried and dehydrated apricots appears to be greater than in peaches or prunes; the tendency is even greater in the case of vitamin A, and points to the probable presence of powerful oxidative catalysts as well as of tissue oxygen in apricots.

P. H. P.

## Bacteriological.

**Limitations of Phenol Coefficients of Coal-Tar Disinfectants.** C. M. Brewer and G. L. A. Ruehle. (*Ind. Eng. Chem.*, 1931, 23, 150-152.)—Experiments with a considerable number of samples of coal-tar disinfectants lead the authors to conclude that it is impossible to calculate the *Staphylococcus aureus* phenol coefficient from the *B. typhosus* coefficient, and that the phenol coefficient is limited in usefulness to interpretations based on comparisons of different disinfectants against test organisms alone and only under prescribed conditions. Any attempt to estimate the efficiency of a disinfectant against other species of pathogenic bacteria from the *B. typhosus* phenol coefficient is unreliable and unsafe. (See ANALYST, 1930, 55, 594.)

W. P. S.

**Inter-relationships of Plankton and Bacteria in Natural Purification of Polluted Water.** C. T. Butterfield and W. C. Purdy. (*Ind. Eng. Chem.*, 1931, 23, 213-218.)—Results of biological and chemical experiments indicate that the chief function of certain plankton in the biochemical oxidation process is to keep the bacterial population of the water below the saturation point, and thus provide conditions suitable for continuous bacterial multiplication, and, as a result, provide for more complete oxidation. This theory of the function of the plankton is supported by the results obtained when the limiting numbers of bacteria were reduced by physical and chemical means. Such reductions were followed invariably by renewed bacterial multiplication and oxidation.

W. P. S.

## Toxicological.

**Hydrogen Sulphide Poisoning.** L. B. Allyn. (*Ind. Eng. Chem.*, 1931, 23, 234.)—A boy who broke off the valve of a cylinder he found on a waste-heap at the bottom of a deep valley was killed almost immediately by the gas which escaped from the cylinder; a rescuer dropped dead as he picked up the body. Other persons were partially overcome by the gas, which was discovered subsequently to be hydrogen sulphide. This tragedy emphasises the poisonous

qualities of hydrogen sulphide in high concentrations; 2000 parts per million cause almost immediate cessation of breathing, and death by acute poisoning is as rapid as in poisoning by cyanides. Hydrogen sulphide is, however, a non-cumulative poison and, if a victim can be revived, there are no systemic sequelae. W. P. S.

**Poisoning by Chloroform during Narcosis. A. Sartori.** (*Chem. Ztg.*, 1931, 55, 222.)—The various organs of a child who died while under the influence of chloroform were examined in the following way:—Of each of the separate organs or mixtures of organs submitted for analysis, either an aliquot part or the whole was mixed with water, and the mixture acidified with tartaric acid and distilled in a current of steam. An aliquot part of each distillate was heated with alcoholic potassium hydroxide and aniline. All gave the odour of isonitrile, which was especially distinct for the brain, stomach, and kidneys. For quantitative purposes, each distillate was heated at 50–60° C., and a stream of air passed through it, the air being then led over glowing glass beads to convert any chloroform present into hydrochloric acid, and afterwards through silver nitrate solution acidified with nitric acid. In no case was any appreciable turbidity thus produced, this result being explained by the interval of 13 days between the death of the patient and the examination of the organs.

The wide variation of the fatal dose of chloroform with different individuals, with the age, with the state of the heart, etc., is emphasised. Whereas many people are able to inhale 50 or 100 grms. of chloroform without suffering harm, as little as 1·5 or 2·5 grms. has sometimes caused death. T. H. P.

**Behaviour of Lead in the Animal Organism. II. Lead Tetraethyl. R. A. Kehoe and F. Thamann.** (*Amer. J. Hygiene*, 1931, 13, 478–498.)—The authors have investigated the rate of absorption of tetraethyl lead through the skin of rabbits, its fate in the tissues over a prolonged period of time, and the rate of its excretion. Full details are given of the manner of applying the lead tetraethyl to the skin of the animals and the collection of the urine, faeces, etc. Lead tetraethyl was extracted from the tissues by distilling in a current of steam in an apparatus permitting bromine to be mixed with the vapour just before it passes into the cooling condenser; the liquid in the receiver then contains the lead as bromide. The residue remaining after the extraction process, and also the urine, etc., was prepared for the lead determination by the usual method of wet oxidation. The actual method of determination of the lead is not described, that given in *J. Amer. Med. Assn.*, 1929, 92, 4, being used. It is shown that lead tetraethyl is absorbed through the skin, but that it is rapidly decomposed by the skin and other tissues, so that only a small portion of the lead found later in the blood is in the form of lead tetraethyl, and no lead remains in this form after from 3 to 14 days. It is concluded that poisoning by lead tetraethyl is not different from lead poisoning by other lead compounds. S. G. C.

## Organic Analysis.

**Saponification Values of Highly Coloured Oils.** H. S. Jois, B. L. Manjunath and S. Venkata Rao. (*J. of Mysore Univ.*, 1930, 4, 241-242.)—The saponification value of highly coloured oils is determined by saponifying 1 to 1.5 grm. of the oil with 0.5 *N* alcoholic potassium hydroxide as usual, and after refluxing for half an hour, adding 50 c.c. of toluene, 25 c.c. of neutral saturated sodium chloride solution, 5 grms. of solid sodium chloride (to prevent dilution of the salt solution during addition of the acid), and lastly, 1 c.c. of phenolphthalein. The liquid is then titrated with 0.5 *N* hydrochloric acid, the mixture being heated on a water-bath towards the end. Since the colouring matter is dissolved in the toluene layer, the end-point is sharp. A blank experiment is made simultaneously and the necessary correction applied. Results obtained by this method with ordinary oils agreed very closely with those obtained by the usual method. The principle has also been applied to the determination of acid values. D. G. H.

**Fluorescence Reaction of  $\beta$ -Naphthol.** N. Schoorl. (*Pharm. Weekblad*, 1931, 68, 279-280.)—A reaction having a sensitiveness of 1:100,000 is obtained if 1 c.c. of a solution containing the  $\beta$ -naphthol is mixed with 5 c.c. of glacial acetic acid, and 5 c.c. of concentrated sulphuric acid added as a layer. The immediate formation of a yellow-green zone, which, on mixing, imparts a green fluorescence to the solution, is a positive reaction (*cf.* Groll, *id.*, 1931, 68, 236). The use of an alcoholic solution of  $\beta$ -naphthol (*cf.* De Haas, *ANALYST*, 1930, 55, 202) is not essential for solutions containing less than 1 part in 2000 (the solubility of  $\beta$ -naphthol in water), but it is necessary to use "ordinary" glacial acetic acid, since acid specially purified by treatment with chromic acid produces a reaction only after some interval (1 to 24 hours). It has been found (*cf.* Zwikker, *Chem. Weekblad*, 1931, 68, 63, 147) that a trace of acetaldehyde increases the rate of reaction with the purified acid, probably in a manner analogous to the Denigès test for aldehydes. J. G.

**Determination of Cellulose.** K. Kürschner and A. Hoffer. (*Chem. Ztg.*, 1931, 55, 161-163, 182-184.)—The results of a number of experiments demonstrate the advantages of a mixture of alcohol and nitric acid as a reagent for obtaining the cellulose of wood free from lignin. The procedure recommended is as follows:—One grm. of the wood (borings) is well mixed with 25 c.c. of the reagent (20 c.c. of 96 per cent. alcohol and 5 c.c. of concentrated nitric acid) in a 250 c.c. flask, which is heated under a reflux condenser on a boiling water-bath for an hour. The liquid is then decanted on to a porcelain or glass filter-crucible, any small quantity of the solid reaching the crucible being rinsed with alcohol-acid mixture into the flask again. The treatment with a total of 25 c.c. of the reagent is repeated, and the mass subsequently filtered; the residue is washed with alcohol and water and very gradually dried, the final temperature being 108° C. The raw cellulose thus obtained is tested for lignins and for pentosans by means of phloroglucinol and hydrochloric acid. If lignins are present, the material is coloured cherry-red

by the cold reagent, which itself remains colourless; if pentosans also are present, subsequent heating colours the reagent red. If, however, as is usually the case, pentosans only are present, no reaction occurs in the cold, whereas, on being heated, the liquid turns red, whilst the solid particles remain practically uncoloured. When lignin is detected in this way, the cellulose is subjected to a third treatment with the alcohol-nitric acid mixture. Of 27 woods of different kinds, none required more than the three treatments.

T. H. P.

**Studies in the Composition of Coal. Methods for the Rational Analysis of Coal.** W. Francis and R. V. Wheeler. (*J. Chem. Soc.*, 1931, 586-593.)—The rational analysis of coal involves the determination of the quantities of free hydrocarbons and resinous compounds, organised plant entities, and ulmin compounds of which the coal is composed, together with a measure of the reactivity of its ulmin compounds (*cf. J. Chem. Soc.*, 1928, 2967). The methods described are used for the routine examination of coals in the Fuel Technology Laboratories of Sheffield University. *Preparation of the Sample.*—The sample of coal is ground, dried, and sieved through a 60-mesh sieve and then on a 120-mesh sieve (I.M.M. standard). Fifteen grms., in a round-bottomed flask, fitted with a ground-in reflux condenser, are boiled in a glycerin-bath with pyridine (225 c.c.) for 8 hours. After cooling, the mixture is filtered through a sintered-glass filter (Schott and Gen., type 17 G.3), and the residue, the amount of which must be determined, washed with a little pyridine; the filtrate is reserved. The extracted coal is transferred to a beaker, boiled for a short time with dilute hydrochloric acid, filtered off, washed, and dried at 105° C.; it is then carefully ground, with frequent sieving, so as just to pass through a 120-mesh sieve; any dust passing through a 150-mesh sieve, the formation of which must be avoided, is discarded. The object of this preliminary treatment is to remove soluble hydrocarbons and resins, and to obtain the same physical condition for all samples. *Hydrocarbons and Resins.*—As much as possible of the pyridine from the above reserved filtrate is removed by distillation, and the residue is boiled for  $\frac{1}{2}$  hour with an excess of dilute hydrochloric acid, cooled, and filtered through a coarse filter paper. The residue, after washing with water, is dried and extracted with ethyl alcohol in a Soxhlet extraction apparatus, the weight of the extracted material giving the combined amount of hydrocarbons and resins in the coal. *Resistant Residue.*—The coal, prepared as described above (0.5 grm.), is boiled for 7 hours under reflux with an oxidising solution, chosen from the following table (p. 334), appropriate to the carbon content of the coal.

The mixture is cooled and filtered through a weighed sintered-glass crucible (2 G.3). The oxidised coal is washed from the filter into a beaker, the volume made up to 100 c.c., 20 c.c. of *N* sodium hydroxide are added, and the mixture gently boiled for  $1\frac{1}{2}$  hours. [The appearance under the microscope of a small sample of the residue remaining after this treatment will indicate whether the correct oxidising solution has been used; a practical trial of possible oxidising solutions may be necessary; if the oxidising solution used is too weak, the resistant residue



Carbon content of coal.*	Oxidising solution.			
	HNO <sub>3</sub> , c.c.		Water, c.c.	ClO <sub>3</sub> , grm.
	N-acid.	2N-acid.		
78	37.5	—	12.5	Nil
79	40	—	10	Nil
80	42.5	—	7.5	Nil
81	45	—	5	Nil
82	47.5	—	2.5	Nil
83	50	—	Nil	Nil
84	46	and 4	Nil	Nil
85	—	28	22	0.2
86	—	29	21	0.35
87	—	30	20	0.5
88	—	32.5	17.5	0.625
89	—	35	15	0.75
90	—	40	10	1.0
91	—	50	Nil	1.5

\* On "pure coal" basis, i.e., after making allowance for the fact that the "ash" of a coal is not of the same composition or quantity as the original mineral matter (see Tideswell and Wheeler, *Amer. Inst. Min. Met. Tech. Paper*, No. 104, 1928).

will be opaque; if too strong, the residue will consist of bleached and macerated fragments, whilst the correct oxidising solution should yield transparent particles of a yellow, orange, or red colour (except those of fusainised wood tracheids), and the larger particles will usually be portions of macrospore exines, and may not be quite transparent.] The solution is filtered through the crucible previously used, with gentle suction, if necessary, and the residue, after having been washed successively with hot water, dilute hydrochloric acid and hot water (3 or 4 times), is dried and weighed. The weight (P) represents the resistant plant entities together with insoluble ash. Further work is necessary to establish the amount of resistant residue in the case of durain coal, for which reference should be made to the original paper. *Reactivity Index of the Ulmins*.—The prepared coal (0.5 grm.), with 0.9 grm. of potassium chlorate and 50 c.c. of N hydrochloric acid, is placed in a 300 c.c. pressure bottle (screw-stoppered, or, for research purposes, a sealed-off flask), which is immersed in a water-bath which is then quickly heated to boiling; the boiling is continued for 7 hours, the flask is allowed to cool overnight, and its contents are filtered through a sintered glass crucible (1 G.3). After washing, the residue is rinsed into a beaker with 100 c.c. of water, 20 c.c. of N potassium hydroxide are added, and the mixture boiled gently for 1½ hours. The solution is filtered through the same crucible, and the residue washed successively with hot water, dilute hydrochloric acid, and hot water, slight suction being used only if unavoidable. The weight (R) of the residue, after drying at 105° C., and its ash content (A) are determined. The weight of ash (A) in 0.5 grm. of the prepared coal is also determined from the data found above.

$$\text{Reactivity Index} = \frac{(0.5 - A) - (R - A_1)}{(0.5 - A) - (P - A_1)} \times 100$$

A table is given of typical rational analyses of British coals.

S. G. C.

**Determination of Water in Vegetable-tanned Leather.** R. F. Innes and J. G. M. Coste. (*J. Inter. Soc. Leather Trades Chem.*, 1931, 15, 126.)—It is shown that when leathers are dried in an air oven or vacuum oven the consequent loss in weight does not represent water alone. With heavily greased leathers any method of determining the water content based on loss of weight on heating gives results much higher than those obtained by prolonged desiccation over calcium chloride or sulphuric acid at laboratory temperature, and it is shown that certain constituents of the grease are lost. Other volatile materials lost on heating are sulphur and essential oils. The official method for the determination of water in leather is unsound, and should be replaced as soon as possible by a method which will distinguish between water and other volatile constituents of leather. R. F. I.

**Determination of Copper in Tanning Extracts.** D. Burton. (*J. Inter. Soc. Leather Trades Chem.*, 1931, 15, 126; *British Section Committee*.)—The Committee has studied three methods for the determination of copper in chestnut extracts, (a) dissolving the ash in acid and depositing the copper on a weighed electrode; (b) comparing the tint produced by adding hydrogen sulphide to a solution of the copper with standard copper solutions; (c) similar to (b), but using sodium diethyl dithiocarbamate instead of hydrogen sulphide. Of these, method (a) is recommended. The ash of 50 grms. of the extract is heated with a few drops of sulphuric acid until it fumes strongly, then with 10 c.c. of 2*N* sulphuric acid. The solution is diluted to 100 c.c., and electrolysed over-night with a current from a 2-volt accumulator, a weighed cathode of platinum foil being used. After washing in water and immersing for five minutes in absolute alcohol, the cathode is dried in an oven and weighed. Results by six analysts varied from 0.0034 to 0.0050 per cent. of copper. R. F. I.

**Identification in Small Samples of Leather of the Different Minerals used in Tanning.** K. Kamfer. (*Mikrochem.*, 1931, 9, 34–37.)—Salts of iron, chromium or aluminium, or mixtures of these, are most commonly used in tanning. *Chromium* is detected, after conversion into chromate, by the diphenylcarbazide reaction. A sample of less than 1 mgrm. is sufficient for the test. The sample is ashed in a porcelain micro-crucible, and then melted with a particle of sodium peroxide to oxidise the chromium to chromate. The mixture is then dissolved in a drop of sulphuric acid (1:5), and 1 to 2 drops of a 2 per cent. alcoholic solution of diphenylcarboxylic acid are added. In the presence of chromium an intense violet colour appears. As only mercury and molybdenum give the same reaction, and these elements are not used for tanning, the reaction is specific for chromium in leather. *Iron* is detected by the thiocyanate reaction after conversion into the ferric state. About 1 mgrm. of leather is ashed and the ash is evaporated with a drop of nitric acid, heated, and a crystal of potassium bisulphate is added. After dissolving in a drop of dilute hydrochloric acid a drop of potassium thiocyanate solution is added. The characteristic red colour indicates the presence of iron. *Aluminium* is detected by the alizarine reaction, or the fluorescent reaction with morin. The sample is ashed, and, as described above, is fused with potassium

bisulphate, then dissolved in a drop of hot water. For the alizarine test a drop of the test solution is placed on a piece of filter paper which has previously been impregnated with potassium ferrocyanide, and dried. Any iron or chromium is retained in the central zone of the drop, whilst the aluminium moves through the capillaries of the paper to an outer circle. A drop of a saturated alcoholic solution of alizarine is placed on the paper, which is held over a bottle of ammonia, and the violet colour of the ammonium alizarinate is visible. When much aluminium is present the red colour of the aluminium alizarinate is also visible, and when less aluminium is present the red colour is visible only after heating, which volatilises the ammonia. For the morin test a drop of the test solution is mixed with two drops of 2 *N* sodium hydroxide solution in a micro-test tube, and centrifuged. The clear solution is acidified with a few drops of 2 *N* acetic acid, and one drop of a saturated solution of morin in methyl alcohol is added. In the presence of aluminium there is a green fluorescence.

J. W. B.

## Inorganic Analysis.

### Simultaneous Volumetric Determination of Lead and Bismuth.

**H. T. Bucherer and F. W. Meier.** (*Z. anal. Chem.*, 1931, 83, 351–361.)—The volumetric application of several precipitation reactions of lead was studied, the end-point being ascertained by filtration of a small quantity of solution and addition of the precipitant. The most sensitive reaction was found to be the formation of lead selenite, a white crystalline precipitate. The weakly acid lead acetate solution is treated with a standard solution of selenious acid at 80° to 90° C., and the filtered portions are tested with the standard solution after addition of a little alcohol. Bismuth is precipitated as selenite in 0.05 to 0.08 *N* nitric acid solution at 70° C.; towards the end the solution is boiled. The two metals can be determined in the same solution by selenious acid precipitation without an intervening filtration; the bismuth is precipitated first from feebly nitric acid solution. When the precipitation is complete, as shown by a filtered test portion, the solution is treated with an excess of sodium acetate and titrated for lead.

W. R. S.

### Volumetric Determination of Iridium. S. C. Woo and D. M. Yost.

(*J. Amer. Chem. Soc.*, 1931, 53, 884–888.)—Chloriridic acid was titrated in 0.1 to 0.2 *N* hydrochloric acid solution with thiosulphate after addition of potassium iodide, benzene being used as indicator; the operation was conducted in conical flasks, and the volume of solution was such that the benzene layer rose into the upper narrow part of the flask, where its colour could be easily observed against a white background. A potentiometric method also was studied. Standardised titanous chloride solution is added very slowly to the 0.1 to 0.2 *N* hydrochloric acid solutions containing 2 to 3 grms. of sodium chloride in a total volume of 50 c.c. At the end-point the potential changes abruptly from 0.65 to 0.4 volt. The results given show a maximum deviation of 0.3 per cent. from the calculated average.

W. R. S.

**Separation of Iron, Titanium, and Aluminium.** E. Schwarz von Bergkampff. (*Z. anal. Chem.*, 1931, 83, 345-350.)—The precipitation of these metals from tartrate solution and their direct determination are described. The weakly acid solution containing 1 gm. of tartaric acid is saturated with hydrogen sulphide, which reduces the iron to the ferrous state. The solution is made ammoniacal and again treated with hydrogen sulphide, the precipitate collected and washed as usual, and converted into ferric oxide by at least half-an-hour's ignition at 850° C. The filtrate is cooled in running water after addition of 40 c.c. of sulphuric acid (1:1); not more than 10 c.c. of the acid should be required to neutralise the ammonia present. The titanium is precipitated in a bulk of 400 c.c. by 6 per cent. aqueous cupferron solution; the precipitate is collected, washed with 10 per cent. hydrochloric acid, and ignited in the usual manner. The filtrate in an 800 c.c. beaker is made ammoniacal, heated to 70° C. (it should remain clear), and precipitated with a 6 per cent. solution of *o*-hydroxyquinoline (5 grms. of base dissolved in 12 grms. of glacial acetic acid and diluted to 100 c.c.). The solution is stirred and kept at 70° C. for some minutes, the precipitate collected in a porous glass crucible, washed with hot water, dried for 2 to 3 hours at 140° C., and weighed ( $Al_2O_3$  factor, 0.1110). Vanadium interferes in this process. W. R. S.

**Volumetric Determination of Selenium.** B. Ormont. (*Z. anal. Chem.*, 1931, 83, 338-339.)—The author questions the reliability of the method proposed by Benesch (*ANALYST*, 1929, 54, 63). W. R. S.

**Determination of Zirconium by means of Selenious Acid.** S. G. Simpson and W. C. Schumb. (*J. Amer. Chem. Soc.*, 1931, 53, 921-933.)—The quantitative precipitation of zirconium by selenious acid, first described by Smith and James (*J. Amer. Chem. Soc.*, 1910, 42, 1764), was submitted to a thorough investigation. A 10 per cent. solution of selenious acid is used as the precipitant; the recovery of zirconium as basic selenite is complete from hydrochloric acid (less than 0.6 *N*) solutions. In presence of sulphuric acid the precipitation is very slow. Alkali chlorides and nitrates do not, but alkali sulphate does, interfere. Sulphate causes incomplete precipitation at the above acidity. Other quadrivalent elements (hafnium, titanium, thorium, ceric cerium) are precipitated like zirconium. Manganese, zinc, cobalt, copper, lead, bismuth, iron, aluminium, and rare earth metals do not interfere; uranium oxide and vanadic acid are adsorbed, but can be eliminated by re-precipitation, the freshly-precipitated zirconium selenite being soluble in 6 *N* hydrochloric acid, which incidentally forms non-adsorbed vanadyl salt by reduction. The effect of titanium and cerium is eliminated by double precipitation from a solution containing hydrogen peroxide, which peroxidises the former and reduces the latter to cerous salt. Thoria, if present, must be eliminated as oxalate, which necessitates the following sequence of operations: solution of the selenite precipitate in 6 *N* hydrochloric acid, treatment with 40 c.c. of hot 10 per cent. oxalic acid filtration, evaporation of the filtrate with strong sulphuric acid to destroy oxalate, precipitation of the zirconia with ammonia, solution of the precipitate in 15 c.c. of hot 12 *N* hydrochloric acid, re-precipitation with selenious acid in presence of hydrogen peroxide. It is recommended

to re-treat the thorium oxalate, *i.e.* by decomposition by heating with strong sulphuric acid, precipitation of thorium hydroxide with ammonia, solution in hydrochloric acid, re-precipitation of the oxalate, and addition of the last filtrate to that from the first oxalate precipitate.

The following procedure separates zirconium from other elements prior to selenite precipitation: the nearly neutral solution (150 c.c.) containing 20 c.c. of 12 *N* hydrochloric acid and 20 of alcohol is heated, diluted to 500 c.c., boiled, and treated with 20 c.c. of 10 per cent. selenious acid. After standing hot for 2 hours, the precipitate is collected, lightly washed, returned with a minimum of hot water, and dissolved by heating with 15 c.c. of 12 *N* hydrochloric acid; hydrogen peroxide (20 c.c. of 3 per cent.), and water to 500 c.c. are added, the solution boiled and precipitated with selenious acid, and the precipitate collected, washed, and returned, as before. The two filters previously used are digested with 40 c.c. of hot 10 per cent. oxalic acid; the pulp is filtered off and washed, the filtrate and washings being added to the zirconium precipitate. The liquid (200 c.c.) is boiled, treated with 12 c.c. of 6 *N* hydrochloric acid, and left in the cold for at least 10 hours, the thorium oxalate filtered off and washed with a solution containing 40 c.c. of 6 *N* hydrochloric, and 25 grms. of oxalic acid per litre. The filtrate is evaporated with 30 c.c. of sulphuric acid. If the oxalate precipitate is large it should be re-treated as prescribed above, and the second filtrate added to the first. Any precipitated selenium is filtered off; small amounts of colloidal selenium in the filtrate may be disregarded. The solution is precipitated with ammonia, the precipitate collected, lightly washed, returned to the beaker, and dissolved by heating with 15 c.c. of 12 *N* hydrochloric acid. Hydrogen peroxide (as before) is added, and the boiling solution diluted to 500 c.c., again precipitated with the same amount of selenious acid. The precipitate is collected, washed with hot water, ignited (finally over a Meker burner), and weighed as  $\text{ZrO}_2$ . If thoria is known to be absent, the first selenite precipitate is simply treated by double re-precipitation in presence of hydrogen peroxide, the filter papers used being ignited and added to the final zirconia.

For the determination of zirconium in its minerals, use is made of a flux consisting of 15 parts of fine sodium peroxide and one of powdered sugar carbon. Twenty-five grms. of the mixture is placed in a 50 c.c. nickel crucible, and 1 gm. of the fine ore powder thoroughly stirred in. A cover of flux is applied, the crucible placed in running water, and ignition started with a glowing fragment of cotton twine, the crucible being immediately covered. The cold mass is transferred to a 1000 c.c. beaker, 300 c.c. of cold water added, which is then heated to boiling; the crucible is rinsed with water. The liquid is diluted to 900 c.c. and heated till clear enough for filtration. The residue is collected and washed, and the filtrate rejected. If much phosphate is present the residue is rinsed back, treated with hydrochloric acid, then ammonia, again collected, ignited, and the fusion repeated. The residue is then dissolved in hydrochloric acid, the solution evaporated with sulphuric acid till it fumes, diluted, filtered, the filtrate precipitated with ammonia, and the precipitate dissolved in 15 c.c. of 12 *N* hydrochloric acid. This solution is ready for selenious acid precipitation.

If earth acids are present, the re-fusion is carried out with potassium carbonate (ANALYST, 1928, 53, 518). The fusion residue is ignited, fused with bisulphate, the sulphate solution precipitated with ammonia, and the precipitate dissolved in hydrochloric acid for selenious acid precipitation. W. R. S.

**Revision of the New Iodimetric Determination of Vanadium in Alloy Steels and Ferrovandium.** W. Werz. (*Z. anal. Chem.*, 1931, 83, 161-164.)—Certain weaknesses in the author's method (ANALYST, 1930, 55, 769) have come to light, and the following revised process is proposed: *Steel*.—From 1 to 3 grms. of steel drillings are heated with 25 c.c. of phosphoric acid (sp. gr. 1.70), and 200 c.c. of water in a 500 c.c. Erlenmeyer flask until dissolved; this takes normally 10 to 15 minutes. The solution is oxidised with nitric acid (sp. gr. 1.4), an excess of 5 c.c. of this acid being added, and boiled for a few minutes to drive out nitrogen oxides. Any insoluble residue of chromium carbide is filtered off and rejected. Ten c.c. of ammonium persulphate solution (5 per cent.) are added, and the solution is boiled for 35 minutes; 25 c.c. of phosphoric acid (sp. gr. 1.70) are then added, and the solution is cooled. From 2 to 10 c.c. of potassium iodide solution (0.2 N) are added (6 c.c. are required for a 3 gm. sample containing about 1 per cent. of vanadium). The solution is kept for 5 minutes, and the liberated iodine is titrated with 0.05 N sodium thiosulphate (1 c.c.=0.00255 gm. V) starch being used as indicator. The volume of the solution titrated should be roughly 200 c.c., corresponding with a phosphoric acid concentration of 1:3; the optimum temperature is 19° to 22° C. *Ferrovandium*.—One gm. is dissolved in 50 c.c. of nitric acid (sp. gr. 1.2). The solution is diluted to 500 c.c., and 50 c.c. of this are withdrawn into a 500 c.c. Erlenmeyer flask, 50 c.c. of phosphoric acid, 150 c.c. of water and 5 c.c. of nitric acid (sp. gr. 1.4) are added, and the process carried out as for steel.

S. G. C.

**Determination of Magnesium in Portland Cement and Similar Materials by 8-Hydroxyquinoline.** J. C. Redmond and H. A. Bright. (*Bureau of Stds. J. Research*, 1931, 6, 113-120.)—The authors have thoroughly investigated the 8-hydroxyquinoline method for the determination of magnesium and its application to the analysis of Portland cement, and propose the following process as an equally accurate rapid alternative to the more usual pyrophosphate method.—The sample (0.5 gm.) is decomposed by gentle heating with a mixture of 10 c.c. of water and 10 c.c. of concentrated hydrochloric acid in a 400 c.c. beaker. Three drops of methyl red indicator (0.2 per cent. in alcohol) are added, and then ammonia solution (sp. gr. 0.9), until the solution is distinctly yellow; some macerated filter paper is added, the solution is boiled for 1 or 2 minutes and the precipitate of alumina, etc., allowed to settle. The precipitate is filtered, without further delay, and washed with hot ammonium chloride solution (2 per cent.). The calcium in the filtrate is precipitated by adding 1 c.c. of ammonia solution (sp. gr. 0.9), heating to boiling, adding 25 c.c. of hot ammonium oxalate solution (4 per cent.) and continuing the boiling for 2 to 3 minutes. The liquid is digested on a steam-bath for  $\frac{1}{2}$  to 1 hour, filtered, and the calcium oxalate washed 5 or 6 times with warm

water. The volume of the filtrate at this point should be about 350 c.c. The filtrate is heated to 60° to 70° C., and 20 c.c. of 8-hydroxyquinoline solution are added (prepared by dissolving 25 grms. of the compound in 60 c.c. of glacial acetic acid and diluting to 2 litres with cold water; 1 c.c. is equivalent to 0.0016 gm. of MgO); this is followed by the addition of 4 c.c. of ammonia solution (sp. gr. 0.9) for each 100 c.c. of solution; the whole is stirred mechanically for 10 to 15 minutes, and then kept until the precipitate has settled. The alternative to be adopted if no mechanical stirrer is available is to heat the solution to 60° to 70° C., and add 4 c.c. of the ammonia solution for each 100 c.c. of solution, and then add 40 c.c. of the 8-hydroxyquinoline solution and heat to boiling, when the beaker is at once set aside until the precipitate has settled. The precipitate, magnesium hydroxyquinolate,  $\text{Mg}(\text{C}_9\text{H}_6\text{ON})_2 \cdot 2\text{H}_2\text{O}$ , is filtered, washed with hot dil. ammonia (1:40) and dissolved in 50 to 75 c.c. of hot dilute hydrochloric acid (1:9); this solution is diluted to 200 c.c., 15 c.c. of concentrated hydrochloric acid added, and cooled to 25° C. The magnesium is now determined by the volumetric determination of the 8-hydroxyquinoline in the solution by the bromate-bromide method as follows:—Twenty-five c.c. of 0.2 *N* bromate-bromide solution (prepared by dissolving 20.00 grms. of potassium bromide and 5.57 grms. of potassium bromate in 1 litre of water) are added, followed immediately by 10 c.c. of potassium iodide solution (25 per cent.); the liberated iodine is titrated with 0.1 *N* sodium thiosulphate solution (standardised against 0.1 *N* iodine solution, which, in turn, has been standardised with arsenious acid), with 2 c.c. of starch solution (0.5 per cent. in 0.25 per cent. aqueous salicylic acid) as indicator. The ratio of the bromide-bromate solution to the standard thiosulphate solution is obtained by adding 25 c.c. of the former to 200 c.c. of water and 20 c.c. of conc. hydrochloric acid, followed by 10 c.c. of the potassium iodide solution and titration of the liberated iodine with the thiosulphate solution. One c.c. of 0.1 *N* thiosulphate is equivalent to 0.000504 gm. of MgO.

S. G. C.

#### Determination of Small Quantities of Boric Acid by a Flame Test.

W. Stahl. (*Z. anal. Chem.*, 1931, **83**, 288–289, 340–344.)—Boric acid in quantities of a few mgrms. cannot be determined accurately by the ordinary volumetric or gravimetric processes. Traces may be determined by Bertrand and Agulhon's colorimetric procedure employing turmeric paper (*Bull. Soc. Chim.*, 1914, **15**, 292). For quantities of the order of 0.3 mgrm., the following process involves errors not exceeding 15 per cent. The vapour of methyl borate is introduced through the air hole of a Bunsen burner, and the intensity of the green coloration compared with that produced by a known amount of boric acid. The apparatus required consists of a set of standard alcoholic solutions of boric oxide, three Bunsen burners of equal size with special fittings, and means for simultaneously passing equal amounts of air through the unknown and two standard solutions. The strongest flame effect is given by a mixture of 100 volumes of methyl alcohol and 20 of strong sulphuric acid. The boric acid to be determined need not be previously separated. The smallest quantity of boric acid which can be detected

by the method by means of a set of micro-burners is 0.005 mgrm. The original papers should be consulted for full details.

W. R. S.

## Microchemical.

### Micro-combustion of Carbon and Hydrogen in Mercury Compounds.

**M. Furter.** (*Mikrochem.*, 1931, 9, 27-30.)—Verdino (*Pregl's Quantitative Organic Micro-analysis*, 1930) found that satisfactory determinations of mercury compounds could be made by using Pregl's universal filling. When Pregl's filling (with lead peroxide asbestos to absorb oxides of nitrogen) was used for the combustion of compounds containing not only carbon, hydrogen and mercury, but also sulphur and nitrogen, the tube, after the combustion, was found to be "poisoned," and although correct hydrogen results could be obtained subsequently with the tube, carbon results were invariably low by 1 to 2 per cent., and continued heating of the tube had no beneficial effect.

J. W. B.

**New Sensitive Reaction of Ammonia.** **K. G. Makris.** (*Z. anal. Chem.*, 1930, 81, 212, 213.)—The addition of a slight trace of ammonia to a solution of silver nitrate and tannin causes the reduction of the silver nitrate to silver. A fragment of cotton-wool is moistened with a few drops of the freshly prepared reagent (5 c.c. of 20 per cent. silver nitrate solution and 1 c.c. of a 5 per cent. solution of tannin), and placed in the mouth of a test tube containing about 1 c.c. of the unknown solution and a few drops of potassium hydroxide solution. The tube is then slowly heated, and in the presence of as little as 5γ of ammonia a reduction of silver is apparent soon after boiling.

J. W. B.

**Micro-Determination of Iodine in Common Salt.** **H. Werner.** (*Z. Unters. Lebensm.*, 1930, 60, 495-500.)—The method described by Winkler (*Pharm. Zentralh.*, 1923, 64, 512; Leitch and Henderson, *Biochem. J.*, 1926, 20, 1003) has been adapted by the author. The salt (50 grms.) is dissolved in water, filtered, 1 c.c. of *N* hydrochloric acid added, and the iodide oxidised to iodate by 1 c.c. of chlorine water. After removal of the excess of chlorine by prolonged evaporation, any insoluble salt is redissolved, the mixture cooled and 5 c.c. of 25 per cent. phosphoric acid added with 0.1 gm. of potassium iodide and 1 c.c. of 1 per cent. starch solution. The resulting iodine ( $\text{KIO}_3 + 5\text{KI} + 6\text{HCl} = 6\text{KCl} + 3\text{I}_2 + 3\text{H}_2\text{O}$ ) is titrated immediately with 0.002 *N* sodium thiosulphate solution, 1 c.c. of which is equivalent to 0.042 mgrm. of iodine. The maximum recorded error is  $\pm 3\gamma$  ( $1\gamma = 0.001$  mgrm.) for 50 grms. of salt containing from 5 to 27γ of iodine. In the presence of more than 1.4 mgrms. of manganese as manganous sulphate, or more than 0.01 mgrm. of iron as ferrous sulphate, a blue colour due to liberation of iodine from the potassium oxide by these salts is obtained immediately, but if 4 drops of 10 per cent. oxalic acid solution are added, the reaction with the manganese is inhibited for 10 minutes, while a similar retardation is obtained for iron if the original solution is acidified with phosphoric instead of with hydrochloric acid. The normal degree of accuracy is, therefore, obtainable by these means in



the presence of either or both of these compounds if the solution is titrated immediately after addition of the reagents, when the iodine from the iodate only is liberated.

J. G.

## Physical Methods, Apparatus, etc.

**New Micro-Balance.** J. Donau. (*Mikrochem.*, 1931, 9, 1-14.)—Certain disadvantages of the Nernst torsion balance, such as the lack of proportionality of the swings, the time taken to come to rest, and the fact that the Nernst balance is built for a fixed tare, have been overcome in the construction of a somewhat similar type of torsion balance. The balance is simple to construct, and can be made of glass or metal (magnalium or duralumin). The balance differs from the Nernst balance in that it has two arms of approximately equal length and the pointer in the centre. The pointer is attached just below the position where the two arms join it, to a fine quartz or tungsten thread, by means of shellac or selenium. The thread is stretched between the ends of an arc-shaped support made of metal, or, better, of quartz or glass, since these are less affected by temperature variations. The weight of the balance is usually 300 to 400 mgrms.; heavier models weighing more than 750 mgrm. show more variation in the zero point, while that of the lighter models stays very constant. The advantage of having two arms is that varying tares may be used, the load ranging from 150 to 400 mgrm. In the glass model both the arms are bent downwards near the point where the pans are to be suspended. The arms of the metal balance are straight, but small screws are placed on the ends, by means of which the centre of gravity may be lowered or raised. By suitably adjusting the angle of bend of the glass model, or the screws of the metal model, by empirically testing the different positions, the balance can be adjusted so that it maintains the same sensitiveness throughout its whole range, and no calibration table is necessary.

Three different models of the balance were used for a number of determinations. The first weighed about 400 mgrms. and one scale division represented 0.059 mgrm. (readings are taken to 1/100 of a scale division). The pointer came to rest in about 6 seconds. The second model weighed about 0.300 mgrm., and one scale division represented 0.0247 mgrm.; readings could be taken after 4 seconds. The third model weighed 800 mgrm., 0.03 mgrm. represented one scale division, and the swinging came to rest in 10 seconds. Excellent results were obtained in test determinations with each of the balances. The same type of balance was used for determinations of small amounts of the heavy metals (*cf.* ANALYST, 1930, 55, 598).

J. W. B.

**Some Applications of Ultra-Violet Light.** L. Colombier. (*Ann. Falsificat.*, 1931, 24, 89-96.)—An unsuccessful attempt was made to determine pH values with certain substances, such as eosin, quinine and acridine, by means of the fluorescence under ultra-violet light. It was found, however, that umbelliferone, in particular, and its derivatives, were excellent indicators with strong acids and bases up to dilutions of 0.001 *N*, also with acetic acid, and with ammonia

to 0.01 *N*. At a *pH* about 6.5 the blue fluorescence suddenly disappears in passing from alkalinity to acidity. Ultra-violet rays, in combination with umbelliferone, allow of the titration of 1 c.c. of acid solution diluted to 250 c.c. with 0.01 *N* alkali, but results are lower than with phenolphthalein, which changes at a *pH* of 9 to 10. Malic acid may be detected by placing a few c.c. of the solution, or particles of the solid, on a watch glass with 1 c.c. of a 0.1 per cent. solution of resorcinol, evaporating the liquid, and taking up the residue with 1 c.c. of sulphuric acid (1 vol. of concentrated sulphuric acid and 1 vol. water). After 5 minutes on a water-bath, 10 c.c. of water are added, and just enough sodium hydroxide to neutralise the acid. If malic acid is present, a blue fluorescence appears when viewed by ultra-violet light. The reaction takes place with all  $\beta$ -ketonic acids, and an analogous and specific reaction occurs with citric acid after oxidation with permanganate. The permanganate is added to the boiling solution until a light yellow colour results, the solution then poured into a crystallising dish with resorcinol, and the procedure carried on as for malic acid, when a violet fluorescence indicates the presence of citric acid. The violet fluorescence produced with resorcinol is specific, but ethyl aceto-acetate is more satisfactory than malic acid in this reaction. A few mgrms. of  $\beta$ -naphthol, dissolved in concentrated sulphuric acid, warmed for 2 minutes and examined by ultra-violet light show a blue-violet fluorescence, and the reaction is specific. A fraction of a mgrm. of orcinol may be detected if Crump's reaction (with chloroform and potassium hydroxide) is observed under ultra-violet light.

D. G. H.

## Reviews.

MONOGRAPHS ON BIOCHEMISTRY. ENZYMES. By J. B. S. HALDANE, M.A.  
Pp. vi+235. London: Longmans, Green & Co. 1930. Price 14s. net.

The present volume is, according to the author, not intended to supersede the previous one of this series, entitled "The Nature of Enzyme Action," by the late Sir Wm. Bayliss. If we understand the author aright, each of these books—the old and the new—is written from the particular point of view of its author.

An enzyme is defined as a soluble colloidal, organic catalyst, produced by living organisms, a definition which rules out crystalloidal catalysts and active surfaces, which appear to be responsible for many oxidations and reductions. Whilst not denying the views of Bayliss and others, that a catalyst can merely accelerate a reaction which occurs slowly in its absence, the author puts forward the less comprehensive idea that a catalyst can promote a reaction only provided that a loss of free energy results. This conception is consistent with the fact that many reactions promoted by enzymes are reversible, for it must be the energy liberated that causes the reversion. The directive power of enzymes is recognised by the author.

A very useful table is given showing the optimum  $pH$  value of the best known enzymes and their sources. The conception that an enzyme unites with its substrate is discussed with the aid of mathematics, and a list of substrate concentrations at which half the maximum initial velocity is reached is given for different enzymes. The value in many cases is identical with the constant of Michaelis.

The course of enzymatic reactions is discussed from the mathematical standpoint. Very comprehensive chapters are those on Specificity, Co-enzymes, Activators, Kinases and Complements, and on the Poisoning of Enzymes.

Due attention is paid to the work of Willstätter and others on the purification of enzymes, and the concluding chapter deals with "The Classification of Enzymes and on Theories of Enzyme Action."

The Bibliography has been carefully prepared, and is comprehensive.

The book is in many respects unique, in that it deals with the subject from a different point of view from that of any other book in the English language, and doubtless it will supply a want.

ARTHUR R. LING.

(1) *UNTERSUCHUNG DER NAHRUNGS-UND GENUSSMITTEL, ALLGEMEINE METHODEN.* Dr. WILHELM PLÜCKER. Urban & Schwarzenberg, Berlin. 1931. Pp. 1046. Price 55 R.M. (59 R.M. bound).

(2) *TABELLEN-UND RECHENBUCH FÜR NAHRUNGSMITTEL-CHEMIKER.* Dr. WILHELM PLÜCKER. Pp. 231. Price 20 R.M. 22 R.M. (bound).

(1) This is an important—and weighty—volume, which is of considerable interest because there is no English work (so far as the reviewer is aware) which covers quite the same ground. It is essentially a treatise on analytical methods applicable to foodstuffs, including oils and all substances coming within the purview of the food chemist; there is no discussion of the composition of particular foods or of limiting values for various constants, but there is a really valuable collection of methods, reactions and processes, together with numerous references. The methods treated of include such matters as sampling, with a simple mathematical treatment of its probable errors, and grinding, and a discussion on the volume corrections for precipitates. There is, too, a useful chapter on biological methods, including the precipitin reactions and fermentation processes as applied to the analysis of sugars and honey.

At the other end of the volume there are working details of physical methods, including cryoscopy, spectroscopy, viscosimetry,  $pH$ , electrometric titrations, and conductivity, in addition to the well-known fundamental operations such as weighing with the microbalance and the usual measurements.

The bulk of the volume consists of a collection of the qualitative reactions of nearly all the various substances which occur in foodstuffs, followed by details of quantitative methods, with many alternative processes suggested for special purposes or applicable to particular commodities. There are useful indications of which method is best for a given purpose, or its limitations in the presence of

possible interfering substances, and methods are given for a wide range of determinations which are not of everyday occurrence, and for which reference to books is likely to be made by the analyst before starting; one can turn to Dr. Plücker and save laborious study of journals.

There are two defects, from the point of view of the English reader, arising mainly from the fact that the subject is treated largely from a biological aspect. The volume appears complementary to part of Abderhalden's *Handbuch der biologischen Arbeitsmethoden*, which is the reason for a number of cross references to alternative processes or details not contained in these covers. The second defect is less excusable—the omission of many valuable methods which have been published in THE ANALYST and other English journals and of mention of processes of great importance in this country. A few examples may be cited in the hope that such omissions may be rectified in a future edition. There is no adequate treatment of the subject of preservatives; sulphur dioxide is not given at all, nor benzoic acid, though there is much information on succinic, malic and other acids. For the determination of fibre, *N* nitric acid is prescribed, whereas in England one must generally use 1.25 per cent. sulphuric acid for official tests. The determination of lactose in milk is given, and of total solids in such products as condensed milk, but no mention is made of the work of the Committee of this Society, nor of Dr. Monier-Williams' contributions. Methods are given for starch determination, but not that by Rask. The alcohols are discussed, but the useful methods given by Adams and Nichols (ANALYST, 1929) are omitted. The acetin method for glycerin assay is mentioned, but not described, though it is used very widely in England and America, much more so than the bichromate method. Truly, Fachini and Somazzi's method is more modern, but it is open to serious criticism. This list might be much extended, but will suffice to indicate that the author has not adequately followed non-German publications, if his work is intended to appeal widely to non-German readers.

Subject to these limitations, the book is to be warmly commended because of what it does include—and that is a vast amount.

(2) This is a companion volume, adding much to the utility of the former. It is not an ordinary book of tables, though it does include most of the usual data, numerical and otherwise. The particular features are detailed tables of special values, such as those of phloroglucides of the less common carbohydrates forming pentosans, Lane and Eynon's and other sugar tables, tables of refractive indexes of solutions of different alcohols, butyric acid values of butter and of coconut-oil mixtures, A. and B. values (Kuhlmann and Grossfeld), and a host of useful matter otherwise only available in the original memoirs.

Although the list of contents is well detailed, an index should be added.

It seems strange to English minds that such valuable—and expensive—books should be sent out in paper covers; anyone purchasing them would do well to buy the bound volumes. The weight is such that, with paper covers, the books would soon fall to pieces.

H. E. Cox.

**THE PRINCIPLES OF PLANT BIOCHEMISTRY.** Part I. By MURIEL WHELDALE ONSLOW, M.A. Pp. 326. Cambridge: At the University Press. 1931. 16s. net.

As stated in the preface, the book deals only with the higher plants, a limitation, which, it is true, keeps the book within reasonable proportions, but, on the other hand, leads to a distorted view of the subject it purports to deal with; in practice it has, however, not been found possible strictly to adhere to this, since the subject of respiration has of necessity required some reference to the behaviour of yeast. The subject-matter is divided into six chapters, devoted respectively to The Sugars, The Cell Wall, Oxidising and Reducing Systems, The Plant Proteins, Nitrogen Metabolism, and Respiration. It is claimed that, as far as possible, each section has been treated comprehensively, and it must be conceded that the author has exhibited remarkable industry in abstracting a very formidable mass of literature and attempting to present the reader with an intelligible account of all this. Each chapter is provided with a useful bibliography which will be welcomed by those in search of first-hand information. The author has throughout endeavoured to stimulate interest by criticising or appraising the value of the researches described, and suggesting in which direction further work is indicated. The criticism is, perhaps, not always quite justified, as in the case of the implied stricture upon the methods of certain authors who "estimate pentoses and pentosans in leaves, without taking into account the presence of uronic acids in pectins, etc. . . ." and this in an alcoholic extract (of leaves) which was subsequently treated with basic lead acetate before analysis.

P. HAAS.

**CHEMICAL METHODS IN CLINICAL MEDICINE.** First Edition. By G. A. HARRISON, B.A., M.D., B.Ch. Pp. ix+534. London: J. & A. Churchill. 1930. Price 18s.

Dr. Harrison is to be congratulated on producing a book greatly wanted in the English language, dealing with the application of chemistry to pathological states.

Many books have been written which deal with sections of this subject, but the comprehensive way in which Dr. Harrison has attacked and successfully carried out his task is worthy of admiration. His aim has been to discuss the chemical nature of pathological states, to equip us with reliable methods of analysis, both qualitative and quantitative, to teach us the deductions to be drawn, and to illustrate his conclusions with typical analyses and case records.

Apart from the value of the book to the medical profession, it should prove of great service to those analysts who are called on to make pathological investigations. They will find full details of chemical processes, and ample references should they desire to consult original publications.

The author, in his experience of teaching, refers very truly to the mystery which surrounds the term  $pH$  in the medical student's mind, and suggests the use, instead, of  $CH$ , *i.e.* the concentration of hydrogen in ionised form. For those whose time only permits of the acquiring of a limited knowledge of chemistry and mathematics, there is a good deal to be said for the employment of this term.

It is pleasing to recognise the caution of the author in his claims for the values of pathological chemistry in diagnosis and treatment; *e.g.*, to quote two instances, the disappointing results of the Van den Bergh reaction in the diagnosis of the nature of jaundice; and the futility, except in one or two special conditions, of the determination of blood calcium. On the other hand, however, it may be suggested that the proved value of the colloidal gold reaction on cerebro-spinal fluid renders it worthy of a more detailed exposition.

Although throughout the book very full and valuable tables of the results of analyses are given, they can be misleading. For instance, on page 390, results of analyses of test meals are given in summarised form. To quote one item as an example, the free hydrochloric acid in cases of duodenal ulcer is given as varying from 0.00 to 0.35 per cent. In this condition the typical feature is hyperacidity, and values of 0.2 per cent. and over will be found in 90 per cent. of the cases, whilst values of 0.00 per cent. will occur about once in 1000 patients suffering from this disease. Thus the bald statement cited above, although strictly accurate, does not stress the characteristic hyperacidity, and will mislead the inexperienced. Similar criticism may be directed to other items.

In the chapter on "Miscellanea and Conundrums" it would have been far better to deal with the detection of blood stains fully or omit the section. It is not in keeping with the thoroughness which characterises the rest of the book. The apology for its inclusion is that the chemical pathologist is occasionally asked to examine blood stains, but surely he is asked more often to make analyses of vomit to determine the nature of the poison in cases admitted to hospital in an urgent state. So far as the commoner poisons are concerned, the author may well consider this inclusion in a future edition.

The book is well printed and bound, and a number of helpful illustrations are included. The book has a satisfactory index.

G. ROCHE LYNCH.

**ELEMENTS OF WATER BACTERIOLOGY.** Fifth Edition. By S. C. PRESCOTT, S.B., Sc.D., and C. A. WINSLOW, S.M., Dr.P.H. Pp. viii+219. New York: John Wiley & Sons; London: Chapman & Hall. 1931. Price 12s. 6d.

The first edition of this American work was issued in 1904, and was a little book of 162 pages of small size; the present edition has 219 much larger pages, a fairly representative increase for a subject which has not shown any very extensive developments of recent years. Sewage and shell-fish were not, however, included in the first edition.

In this space the authors have well covered their subject. After a short chapter on the bacteria in natural waters, follow three chapters on the quantitative examination and its interpretation. Counts on both gelatin and agar are recommended, but no reference is made to the use of agar for both low and blood temperature counts, a procedure of value. As regards the composition of the media the authors take the sound view that the aim is not media which yield the maximum numbers, but rather that the object is to bring out the quantitative differences

between potable and polluted waters. Throughout, the recommendations of the Standard Methods Committee of America are quoted and generally accepted. The English Standardisation Committee's Report of 1904 is mentioned, but not their second report.

Two chapters are devoted to the colon group, and the significance to be attached to the presence of these strains. While the value of the test is emphasised, the important question of permissible standards is not clearly treated, and the directions are difficult to follow and to use. The greatest divergence from English methods is in regard to the failure to recommend bile salt media for the estimation and isolation of this group. Experiments, using bile itself, are given at some length, and their value recognised, but the authors apparently acquiesce in the views of the Standards Committee, which make no mention of bile, and recommend ordinary lactose broth as the enrichment medium. Lactose bile salt neutral red agar is not even mentioned, and there is considerable discussion as to the relative value of Endo agar and eosin methylene blue agar. English bacteriologists are so satisfied with the use of the bile salts broth and agar that there is no need to try others, and most of them gave up Endo agar many years ago. There is an interesting discussion on the relative value of the *B. coli* and *B. aerogenes* groups, the authors taking a rather non-committal attitude as to the value of their differentiation in judging the potable qualities of a water.

The utility of *B. enteritidis sporogenes* and streptococci determinations are discussed, but little value is placed upon their estimation. The former is stated to be now called *B. sporogenes*, but the usual view is that the characteristic milk change is due to *B. welchii*, Klein's original strain being a mixture of anaerobes.

It is refreshing to note that, unlike so many recent American books, credit is given to English work, much of which was pioneer work, although some important English investigations are omitted. For example, Wilson's recent work on the isolation of *B. typhosus* and *B. paratyphosus* from sewage is not mentioned, while standard English methods for examining shell-fish are ignored.

The book is clearly written, well printed, and contains a great deal of valuable information, while the teaching is mostly in line with English experience. With a few reservations, some of which have been indicated, it can be recommended as a reliable guide to the subject and as a most useful book of reference.

W. G. SAVAGE.

PERFUMES, COSMETICS AND SOAPS, WITH SPECIAL REFERENCE TO SYNTHETICS.

By W. A. POUCHER. Third Edition. Vol. I. Pp. xxix+394, with 30 illustrations. Vol. II, pp. xiv+521, with 66 illustrations. London: Chapman & Hall. 1930. Price 21s. and 25s. net, respectively.

The popularity of this work is shown by the fact that though only first published in 1923, a third edition is already called for.

Vol. I, which maintains its character as a dictionary of raw materials of value and interest to the chemist-perfumer, has been expanded by the inclusion of some

30 more natural products, and about 250 new synthetics, making, in all, a total of over 1000 substances dealt with. The value of the volume to the analyst is much increased by the inclusion of the chemical formulae and physical constants for nearly all the synthetics, and the very large number of formulae for the reproduction of natural perfumes by mixtures of synthetics is still a very useful feature of the book.

It is a pity that advantage has not been taken of the issue of a new edition to correct some of the errors referred to in the review of the second edition (ANALYST, 1926, 51, 275), but except for the corrected spelling of safrole, these all remain. Under zinc stearate it is interesting to note a warning that powders containing this salt have been reported in the U.S.A. to have caused illness and even death in children, and the use of magnesium stearate instead is recommended.

Vol. II has been enlarged by the inclusion of four new chapters dealing with (1) the Purchase and Use of Flower Absolutes, (2) Odour Classification, (3) Sachets and Solid Perfumes, and (4) Hair Dyes. Among the last-named, paraphenylenediamine is said to be the most important, and the dangers attending its use are pointed out.

In this volume, also, no attempt has been made to correct the defects referred to in the review of the second edition (ANALYST, 1927, 52, 109), except that the subject of transparent soap manufacture has now been much expanded; but here, however, there is another mis-statement, namely, that in the process of dissolving soap in alcohol and distilling off the solvent, the soap should be "a first class milling base," whereas it is customary to use a tallow-rosin (primrose) soap, the rosin playing an important part in securing transparency.

Most of the very large number of formulae included have been revised, and brought into accord with present-day practice.

The work is written in a concise but interesting manner, and is well illustrated, and this edition will doubtless prove as popular as the earlier ones have done.

W. H. SIMMONS.

ANALYTICAL CHEMISTRY. Vol. I. QUALITATIVE ANALYSIS, BASED ON THE GERMAN TEXT OF F. P. TREADWELL. Translated and Revised by WILLIAM T. HALL. Seventh English Edition, Revised. Pp. ix+610. New York: John Wiley & Sons, Inc.; London: Chapman and Hall, Ltd. Price 23s.

The new edition of Treadwell-Hall's Qualitative Analysis has undergone a few changes, some of which will be discussed below; the text-matter on the Rare Earths has been altered with the help and advice of Mr. H. F. V. Little, author of the scholarly treatise on Aluminium and its Congeners, in Newton Friend's Series of Text-books.

The book is so well known that a general survey of its contents has become superfluous. I can, therefore, confine my attention to certain parts, dealing mostly with rarer elements.



An example of how errors are perpetuated in text-books is furnished by the familiar chart showing the spectra of a number of metals. While still at school I was an assiduous reader of Wislicenus's Treatise, published about 1875. The old book and the one under review contain the same chart, in which the wrong symbol, "Ka" for potassium occurs three times.

On account of the high price of platinum, if for no other reason, it would be advisable to mention the use of silica crucibles for pyrosulphate fusions (p. 138). On the same page, we find that the destruction of tartaric acid, etc., is regarded as necessary prior to the detection of aluminium in solution. This is no longer true, as tannin precipitates the metal quantitatively from tartrate solution. Though not specific for aluminium, the reaction might be with advantage described, as it is very delicate, and forms part of a useful and convenient method for the separation of aluminium from iron (ANALYST, 1929, 54, 712).

An unfortunate mistake has spoiled the description of the method for the separation of the earth acids from titania and zirconia (ANALYST, 1929, 54, 454). The colour of the tannin complexes of tantalum and niobium is given as "sulfur-yellow" (p. 168B); actually the colours are sulphur-yellow for tantalum and red for niobium, as stated in the original. As to the application of the procedure, the book directs the operator to "repeat the treatment if the residue is not light yellow in color." Nothing could be more unlike the original text, which gives unconditional directions for a single treatment, while the colour of the residue is given as "buff to bright scarlet." As they stand, the directions are altogether misleading.

A drastic revision of the pages on tantalum and niobium (527 to 531) is badly needed. Much of the text-matter is out of date; some of the reactions are of no diagnostic value; and several statements are erroneous. Sodium hexaniobate has not been isolated; the stable salt is  $7\text{Na}_2\text{O} \cdot 6\text{Nb}_2\text{O}_5$ . Of the tantalum reactions cited, the first consists in the precipitation of tantalic acid by mineral acids from alkaline solution; yet reactions 2, 3, 4, and 8 are described as taking place in acid solution. The following is a glaring instance of careless proof reading coupled with inadequate treatment of the tannin reaction, the most important one in earth-acid analysis. Under Tantalum, we read that "Tincture of nutgalls produces no precipitate (difference from niobic acid)." Turning to niobium, we find that "Tincture of nutgalls produces no precipitate"!

In view of the minute quantities in which rubidium and caesium are met with, the spectroscope is likely to remain the analytical weapon of paramount importance for their detection. It is, therefore, disappointing to find that the spectroscopic examination of the alkali group has been discarded in favour of a wet separation method of Noyes and Bray. The advisability of this change is more than questionable. The wet process represents an achievement involving a disproportionate expenditure of time and labour, and requiring a number of unusual reagents. One may be permitted to question its practical value; it may, no doubt, serve as an exercise for college students working with artificial mixtures

of high rare-alkali content. But no chemist in actual practice, I venture to say, would engage in work of this kind without a spectroscope.

The concluding 20 pages of the book consist of a tabulated outline of Noyes and Bray's comprehensive scheme for the detection of all the known metals. It would be of interest to the profession to know whether the scheme has found practical application outside those colleges where it forms part of the laboratory discipline. I believe, rightly or wrongly, that it suffers from over-elaboration, because it sets out to solve an artificially created problem. Fortunately for the analyst, there is such a thing as paragenetic incompatibility, by virtue of which *substantial* amounts of certain metals (such as would respond to the tests when one grm. of substance is taken as directed) are not met with in association with certain other metals. For example, it is unnecessary to look for metals of the platinum group as major constituents of silicates or titanoniobates. For the detection of traces of an element, on the other hand, it will still be necessary to isolate it from a larger amount of material by a special procedure.

The number of misprints noticed in the book indicates rather superficial proof-reading. A bad slip occurs on p. 168B, line 4 of paragraph 9a, where "tantalum" should be substituted for "zirconium" in the sentence "differs from zirconium and columbium." On p. 499, rubidium chloroplatinate is described as a white crystalline precipitate, and "cerium" occurs twice as a mis-spelling for "cesium."

W. R. SCHOELLER.

IMPURITIES IN METALS. THEIR INFLUENCE ON STRUCTURE AND PROPERTIES.

By COLIN J. SMITHELLS, M.C., D.Sc. Second Edition. Roy. 8vo. Pp. xiii+190, with 181 illustrations. London: Chapman & Hall, Ltd. 1930. Price 18s. net.

The fact that a second edition of this work has been called for within the short space of two years indicates that a very real demand existed for a book which at the time of its first appearance was somewhat novel in conception. The new edition has given the author an opportunity of carrying out a thorough revision of the data presented, and of bringing the whole up-to-date.

An exact knowledge of the influence of impurities, or, as they may preferably be called, "minor constituents" (for they include both accidental and intentional additions), is of interest, not only to the metallurgist and engineer, but also to the physicist engaged on the measurement of fundamental constants of materials, and to the analytical chemist. The subject is here treated as a natural development of metallography. To those who make no special claim to metallographic knowledge the first four chapters will be found to be of considerable value. They deal with methods of studying the Structure of Metals, X-rays and the Structure of Metals, the Structure of pure Metals and the Structure of Alloys.

The general arrangement of the book remains unchanged. The minor constituents are grouped together and classified as metallic, non-metallic and gaseous, with a further sub-division according to their solubility in the metal or alloy.

This method adds to the lucidity of the treatment and stimulates interest by revealing the scientific principles governing the wealth of information presented. It has the effect, however, of scattering the references to the "major constituent" from one end of the book to the other. Thus the chemist, using the book as a work of reference, will find that the effect of impurities on copper is dealt with at frequent intervals between page 3 and page 156. It must be added that this unavoidable inconvenience is compensated for by a useful index; though here, as in the text, the author occasionally trips over names, *e.g.* Andrews for Andrew, Beckinsdale for Beckinsale, Langenburg for Langenberg, Stoke's law for Stokes' law. There still remain a few discrepancies which, however, are not likely to be seriously misleading; for example, the solid solubility of copper in silver is given on p. 44 as 4 per cent. and on p. 116 as 6 per cent., whereas it is actually about 1.7 per cent. (Hansen).

Special attention has been devoted to the revision of the sections on the effect of gases on metals and on the influence of minor constituents on the mechanical properties of metals, as the results of many new researches in these fields have become available since the date of original publication of the book. The production of an exhaustive account of these subjects in the space available would be impossible, but the author has made a well-balanced choice of material, and covers a wide field with commendable accuracy.

The book is amply illustrated, and the excellence of the printing and of the reproduction of the photomicrographs reflect great credit on the publishers.

R. H. GREAVES.

**DIE SCHLÄMMANALYSE.** By Dr. HERMANN GESSNER. (Kolloidforschung in Einzeldarstellungen, Bd. X.) Pp. vii+244. Leipzig: Akademische Verlagsgesellschaft. 1931. Price: Stitched, M.16.50; bound, M.18.

This well-produced monograph deals with the subject of analysis by elutriation, *i.e.* the determination of grain-size of muds, slimes, soils and fine suspensions generally. To the student of soil physics it has special appeal, but it deserves the attention of all interested in the wider field of colloid physics.

Size-frequency analysis is becoming increasingly important, and Dr. Gessner's treatment can readily be adapted to emulsions.

The author commences with Stokes' formula and its recent modifications, following then with an excellent account of modern work on coagulation. The various methods suggested and applied in analysis by elutriation are next examined in considerable detail, with a remarkable array of illustrations of apparatus. These, with graphs, total 103 figures. A critical examination of the value of such analyses closes the volume.

The German is unusually easy to follow, and the printing and binding are excellent. Altogether the book can be heartily recommended to all interested in this special field. The well-known authority, Professor Georg Wiegner, has contributed an appreciative foreword.

WILLIAM CLAYTON.

**Erratum.**—In the review on "Practical Physical Chemistry" (p. 276, line 34) for "27.30" read "27.83."



# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, May 6th, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—Raymond Merefield Edwards, B.Sc., Llewelyn John Howells, B.Sc., Donald Neil McArthur, D.Sc., Ph.D., F.I.C., F.R.S.E., James Sword, M.A., B.Sc., Ph.D., A.I.C.

Certificates were read for the second time in favour of:—George Brown, A.I.C., Charles Loudon, B.Sc., A.I.C., Charles Percy Money, B.Sc., F.I.C., Martin Priest, F.I.C., Arthur Goodyear Simpson, M.A., Gerrish Smith.

The following were elected Members of the Society:—K. N. Bagchi, B.Sc., M.B., D.T.M., William Nelson Bradshaw, B.Sc., Adrian Joseph Clifford Lickorish, F.I.C., Ernest Grenville Purser, B.Sc., A.I.C., and William Waddell Robson.

The following papers were read and discussed:—"A Demonstration of a New Development in Filter Papers," by E. J. Guild; "The 'Rope' Spore Content of Flour and its Significance," by A. J. Amos, B.Sc., A.I.C., and D. W. Kent-Jones, Ph.D., B.Sc., F.I.C.; "The Separation of Tin from Tantalum and Niobium," by W. R. Schoeller, Ph.D., and H. W. Webb (*Work done under the Analytical Investigation Scheme*); "A New Method for Detecting Decomposition Products in Anaesthetic Chloroform," by N. L. Allport, A.I.C.; and "Contaminations in Morphine Deposited in the "British Pharmacopoeia" Process for the Analysis of Opium," by J. N. Rakshit, F.I.C.

## Obituary.

### MEREDITH WYNTER BLYTH.

MEREDITH WYNTER BLYTH was born at Worcester on December 28th, 1871, and died at Tankersley, near Barnsley, on March 31st, 1931.

He was the son of Dr. Wynter Blyth, an original member of the Society, whose well-known works, "Poisons, their Effect and Detection," and "Foods, their Composition and Analysis," he revised and largely rewrote.

He was educated at King's College School and St. John's College, Cambridge, where he gained a double first in the Natural Science Tripos. After leaving Cambridge, he took his Science Degree at London University, and became Gas Examiner for the London County Council, later working as assistant to Klein, at St. Bartholomew's Hospital.

He joined the Society of Public Analysts in 1902, and served on the Council in 1906-7. His contributions to *THE ANALYST* included papers on the detection of preservatives and colouring matters in milk, and on the chemical control and standardisation of disinfectants. In 1896 he was elected an Associate of the Institute of Chemistry, and became a Fellow in 1899.

In 1898 Wynter Blyth started in private practice as a consulting chemist and bacteriologist, and for over 20 years was Public Analyst for the boroughs of Brighton and Eastbourne. In 1905 he joined the staff of Newton, Chambers & Co., Ltd., near Sheffield, as Chief Chemist. He carried out much of the early work on the bacteriological standardisation of disinfectants, and he contributed very largely towards raising their reputation to that in which they are held to-day.

Wynter Blyth was a man brilliant in argument, both in speech and in writing. He had a very keen sense of humour and a ready tongue, and his kindness of heart, his sociability, and his prowess at games, which commenced by his representing his college at lawn tennis with the Doherty brothers, made him universally popular.

W. NEWTON DREW.

## The Rapid Determination of Solid Saturated Fatty Acids.

By T. P. HILDITCH, D.Sc., F.I.C., AND J. PRIESTMAN, Ph.D.

*(Work done under the Analytical Investigation Scheme.)*

*(Read at the Meeting of the North of England Section, February 14, 1931.)*

WE have had under consideration for some time the methods available for determining rapidly the amount of saturated fatty acids in the mixed fatty acids of natural fats, and, in connection with this subject, the Analytical Investigation Committee of the Society suggested that, possibly, an accurate measure of the "solid" unsaturated acids in hydrogenated fats might be obtained by a combination of the lead salt (Twitchell) separation with an oxidation method such as that of Bertram. Subsequently, however, we learned that Mr. L. V. Cocks and his colleagues, in the Research Laboratories of Messrs. Lever Brothers, Ltd., were making a comprehensive investigation of modifications in the Twitchell lead salt separation which

should permit of the determination of "solid" unsaturated (iso-oleic) acids as well as of saturated fatty acids, and we accordingly confined our attention mainly to the determination of saturated acids in non-hydrogenated fats. During the later stages of the work the results obtained by Mr. Cocks and his colleagues and by ourselves have been mutually discussed from time to time. The respective investigations have, of course, been undertaken quite independently by each group, working under different auspices, and to a minor extent, from not quite the same standpoint; but the researches are complementary, and it is, therefore, appropriate that the results should be presented concurrently, and considered in conjunction with each other.

The methods available for the estimation of saturated higher fatty acids depend either on the relative insolubility in different solvents of certain of their metallic salts, or on removal of all unsaturated acids by oxidation. It is proposed to review each of these classes of procedure, but, at the outset, we would point out that no existing method is applicable to the whole range of fatty acid mixtures found in natural fats, and that the type of fat under examination must be considered in every case. Furthermore, it is desirable to consider the probable limits of experimental error liable to be encountered. From the descriptions of some workers it would appear that results can be duplicated and an accurate estimate given for the content of saturated acids to within a few tenths per cent.; in our opinion, in the majority of cases, the order of experimental error is more probably in the neighbourhood of  $\pm 1.0$  unit per 100 parts of total mixed fatty acids, whilst in cases in which saturated acids containing 14 carbon atoms or less per molecule or certain unsaturated acids such as erucic, petroselinic or other solid oleic acids are present, an accuracy of  $\pm 2-3$  units per cent. is the most that can be expected.

In the course of our work we have considered both the lead salt (alcohol) separation and the oxidation processes, and these will be dealt with consecutively.

SEPARATION OF SATURATED FATTY ACIDS AS METALLIC (LEAD) SALTS.—This method has, of course, been available since Gusserow (*Annalen*, 1828, 27, 153) and Varrentrapp (*ibid.*, 1840, 35, 196) drew attention to the differing solubility of the lead salts of the higher saturated and unsaturated acids in ether. Many modifications of their original process have been proposed,\* some of which advocate the replacement of ether by other organic solvents, whilst others have suggested the use of salts of univalent metals such as lithium or thallium. The employment of a univalent metal would be expected to promote separation of saturated from unsaturated acids owing to the impossibility of obtaining mixed salts containing saturated and unsaturated acids in combination with one and the same basic group; but the general experience has been that, even with the thallos salts proposed by Meigen and Neuberger (*Chem. Umschau*, 1922, 29, 342) and by Holde, Selim and Bleyberg (*Z. deutsch. Oel u. Fett-Ind.*, 1924, 44, 277, 298), the separation is not more satisfactory than with lead salts. Presumably, the solubility

\* A bibliography is given by Bertram, *Z. deutsch. Oel u. Fett-Ind.*, 1925, 45, 733.

relationships of such salts of oleic acid and the related saturated acids are too closely alike for separation to be effective in these cases.

It is generally agreed, at all events, that the separation of the lead salts from solution in 95 per cent. alcohol at about 15° C. on the lines recommended by Twitchell (*J. Ind. Eng. Chem.*, 1921, 13, 806) gives the best results. This author recommends adding, to a solution of mixed fatty acids containing 1 to 1.5 gm. of saturated acids dissolved in boiling 95 per cent. alcohol (30 ml.), a solution of lead acetate (about 1.5 gm.) in boiling 95 per cent. alcohol (70 ml.), cooling the mixed solutions slowly to 15° C., and leaving them over-night; the separated lead salts are filtered off, washed with 95 per cent. alcohol until, on dilution, the washings remain clear, and are then recrystallised from 95 per cent. alcohol (100 ml., containing 0.5 gm. glacial acetic acid). The cooling process is repeated, and eventually the separated, washed lead salts are converted by acidification with nitric acid into the free acids, in which form they are weighed.

The proportion of alcohol employed in the first precipitation per unit weight of mixed fatty acids may thus vary, according to the expected percentage of saturated acids in the latter, from about 10 to 30; for mixed fatty acids containing about 30 per cent. of saturated acids it is about 20. Again, whilst Twitchell mentioned the use of sufficient lead acetate to interact with all the fatty acids present, he was inclined to recommend that the lead acetate should be taken so as to be only somewhat in excess of that required for combination with the saturated acids; later workers have reverted to some extent to the use of larger proportions of lead acetate, and in a recent communication describing the application of the Twitchell method to directly saponified fats Baughman and Jamieson (*Oil and Fat Ind.*, 1930, 7, 331) advocate the employment of 5 grms. of lead acetate per 1 to 1.5 gm. of solid acids present in the original fat under test.

The important point to be considered is the extent to which saturated acids may pass into the soluble lead salt fraction and, during a somewhat extensive experience of a modified form of the Twitchell method as a preliminary to fractional distillation of the methyl esters of the "solid" and "liquid" acids so obtained, we have formed the opinion that appreciable quantities of myristic, and still more of lower, acids may find their way into the soluble lead salts or "liquid" acid portion. Further, but to a much less degree, lead palmitate and even lead stearate may, in particular cases, be found with the unsaturated acids in the soluble lead salt fraction.

This may be illustrated by typical examples from the numerous detailed analyses of natural fatty acid mixtures in our laboratory records (which have, with a few exceptions, already been published in various communications). For the fractionation analysis, it is found most suitable to employ, per unit of mixed fatty acids, 10 parts of 95 per cent. alcohol and 0.7 part of lead acetate, and, in some cases, the first crop of "solid" acids has been submitted to a repetition of the lead separation. Table I shows the respective proportions of lauric and lower acids

("C<sub>13</sub> and below"), myristic ("C<sub>14</sub>"), palmitic ("C<sub>16</sub>"), stearic ("C<sub>18</sub>"), palmitoleic or lower unsaturated acids ("lower unsaturated"), oleic and linoleic ("C<sub>18</sub> unsaturated"), C<sub>20</sub> or C<sub>22</sub> unsaturated acids ("higher unsaturated") and unsaponifiable matter estimated by fractionation analysis in the "solid" (S) and "liquid" (L) acid groups.

TABLE I.

Mixed acids of:		C <sub>13</sub> and below.	C <sub>14</sub> .	C <sub>16</sub> .	C <sub>18</sub> .	Lower unsat- urated.	C <sub>18</sub> unsat- urated.	Higher unsat- urated.	Unsap.
Butter* (a)	{ S	1.0	8.5	26.1	6.5	—	12.8	—	—
	{ L	5.9	1.9	—	—	—	32.2	—	0.4
Butter* (b)	{ S	0.9	6.3	27.3	11.5	—	5.9	—	—
	{ L	7.0	4.8	—	—	—	29.9	—	0.3
Sperm head oil	{ S	11.1	11.5	8.0	1.7	9.7	8.3	1.5	0.5
	{ L	8.3	2.0	0.3	—	23.5	8.4	5.0	0.2
Laurel fat	{ S	23.5	—	8.8	—	—	15.4	—	3.0
	{ L	8.2	—	—	—	—	34.5	—	6.6
Nutmeg butter	{ S	1.0	60.1	8.2	—	—	1.7	—	—
	{ L	0.2	1.5	—	—	—	7.8	—	19.5
Palm oil (a)	{ S	—	0.3	39.7	4.3	—	2.1	—	—
	{ L	—	0.9	3.1	0.1	—	49.2	—	0.3
Palm oil (b)	{ S	—	1.5	40.1	3.5	—	2.4	—	—
	{ L	—	0.9	0.7	—	—	50.7	—	0.2
Stillingia tallow	{ S	—	1.7	62.8	1.2	—	0.5	—	—
	{ L	1.9	2.0	3.4	—	—	26.4	—	0.1
Cacao butter	{ S	—	—	21.0	34.4	—	2.1	—	—
	{ L	—	—	4.7	—	—	37.6	—	0.2
Borneo tallow	{ S	—	—	18.5	36.8	—	1.9	—	0.2
	{ L	—	1.4	2.7	2.2	—	35.7	—	0.6
Mutton tallow	{ S	—	0.1	24.1	30.5	—	7.6	—	—
	{ L	—	4.5	0.5	—	—	32.7	—	—
Beef tallow (a)	{ S	—	0.4	30.6	19.1	—	3.9	—	—
	{ L	—	4.0	—	—	—	41.8	—	0.2
Beef tallow (b)	{ S	—	1.2	27.3	14.1	—	3.9	—	—
	{ L	—	5.1	0.1	—	—	48.0	—	0.3
Olive oil	{ S	—	—	9.6	1.9	—	4.4	—	—
	{ L	—	1.1	—	—	—	82.2	—	0.8
Cod-liver oil, Scottish	{ S	—	2.5	9.9	—	4.5	10.2	19.4	—
	{ L	—	1.0	0.3	—	11.3	14.3	25.6	1.0
Cod-liver oil, Newfoundland	{ S	—	3.0	7.3	0.6	1.2	4.4	4.6	—
	{ L	—	2.8	1.1	—	19.0	24.7	30.4	0.9

\* Analyses on mixed fatty acids after removal of volatile acids by prolonged steam-distillation (cf. Hilditch and Sleightholme, *Biochem. J.*, 1930, 24, 1098).

Since, for the purpose of fractionation analyses, the quantities of acids submitted to the lead salt separation are of the order of 100–200 grms., it is not to be expected that the latter can be regulated so exactly as in the actual Twitchell analysis on about 5 grms. of mixed acids; nor is this essential for the purpose of ester analysis by the fractionation method. Apart from this, however, Table I



demonstrates that, when using a ratio of fatty acid: alcohol of 1:10, saturated acids may pass into the soluble lead salt portion somewhat as follows:—

(i) Stearic acid is practically always absent from the "liquid" acids.

(ii) Palmitic acid is usually absent or only present in traces in the "liquid" acids, but has been occasionally observed in more appreciable amounts; this is usually associated with a fatty acid mixture (cacao butter, Borneo tallow, etc.) rich in palmitic and stearic acid, but with a comparatively low oleic acid content.

(iii) Myristic acid usually passes to a considerable extent into the soluble lead salts. The solubility of lead myristate in the alcoholic solution of the soluble lead salts appears to be affected by the composition of the particular mixture of fatty acids present.

(iv) Saturated acids lower than myristic, of course, give lead salts which are increasingly soluble in 95 per cent. alcohol.

In order to throw further light on the above data we have made some tests of the analytical lead salt separation, as described by Twitchell (*loc. cit.*), on the mixed acids of a beef tallow, of olive oil, a Newfoundland cod-liver oil, cottonseed oil and palm oil, mainly employing 1.5 grm. of lead acetate per 1–1.5 grm. of saturated fatty acids.

The composition of the fatty acids in each of these oils had been previously estimated by other workers in this laboratory by the fractionation method (with, we believe, a general order of accuracy of within 1 unit per cent., except in the case of the unsaturated acids of cod-liver oil) with the following results:—

TABLE II.

Acids of:				Beef tallow.	Olive oil.	Cod-liver oil.	Cottonseed oil.	Palm oil.
Saturated:								
Myristic	..	..	..	4.5	1.1	5.8	3.3	2.5
Palmitic	..	..	..	30.6	9.6	8.4	19.8	40.8
Stearic	..	..	..	19.0	1.0	0.6	1.3	3.5
Arachidic	..	..	..	0.1	0.9	—	0.6	—
Unsaturated:								
Below C <sub>18</sub>	..	..	..	—	—	20.2	—	—
C <sub>18</sub>	..	..	..	45.6	86.6	29.1	74.2	53.0
Above C <sub>18</sub>	..	..	..	—	—	35.0	—	—
Unsaponifiable	..	..	..	0.2	0.8	0.9	0.8	0.2
Saturated acids per cent.				54.2	12.6	14.8	25.0	46.8

The results of typical determinations by Twitchell's method, as given in his original paper and summarised on p. 356 of this communication, are given in Table III. Slight modifications in the various experiments are indicated by the appended footnotes. The weight of solid acids obtained was corrected for unsaturated acids on the assumption that the observed iodine values were due only to oleic acid; reference to Table I will show that a considerable proportion of the "solid" unsaturated acids from cod-liver oil is made up of acids of the C<sub>20</sub> and C<sub>22</sub> series, so that in this instance the correction is only approximately valid.

TABLE III.

Mixed fatty acids. Grms.	Acids from insoluble lead salts.			Saturated acids. Per Cent.
	Grm.	Iodine value.	Per Cent.	
(i) Beef tallow.				
(a) 3.174	1.597	3.8	50.3	48.2
(a) 2.521	1.255	4.2	49.8	47.5
(a) 3.004	1.520	4.8	50.6	47.9
(b) 2.817	1.590	9.5	56.4	50.4
(b), (e) 2.784	1.445	7.7	51.9	47.5
(ii) Olive oil.				
(a) 5.560	1.050	37.4	18.9	11.0
(a) 5.945	0.833	17.1	14.0	11.3
(b), (c) 10.025	1.138	2.2	11.35	11.1
(b), (d) 4.933	0.615	8.4	12.5	11.3
(b), (e) 4.837	0.545	10.8	11.4	10.0
(iii) Cod-liver oil (Newfoundland).				
(a) 6.298	1.070	27.5	17.0	11.8
(a) 5.476	0.785	10.5	14.3	12.6
(iv) Cottonseed oil.				
(b) 4.562	1.066	2.8	23.4	22.7
(b) 5.261	1.214	3.0	23.1	22.3
(b), (e) 4.315	0.975	8.4	22.2	20.1
(v) Palm oil.				
(b) 2.973	1.381	2.9	46.5	45.1
(b) 2.743	1.269	1.7	46.2	45.3
(b), (e) 2.652	1.204	3.0	45.4	43.9
(b), (e) 2.865	1.295	1.8	45.2	44.3

(a) Alcohol solutions cooled on bench overnight; temperature not likely to have fallen below 12–15°C.

(b) Alcohol solutions cooled in cupboard overnight and minimum temperature observed to be above 15°C.

(c) Ten grms. olive oil, *i.e.* ca. 1.2 grm. saturated acids, with 1.5 grm. lead acetate.

(d) Five grms. olive oil with 0.7 grm. lead acetate.

(e) Lead acetate equal to weight of total acids, *i.e.* 140 per cent. of theoretical for combination with all the acids.

These figures confirm the view that Twitchell's method, as originally described, frequently gives rather low values for the content of saturated acids, whilst the iodine values of the separated acids may be high when the original mixed acids contain a preponderance of unsaturated acids (this is not necessarily a detriment so long as absence of linoleic acid from the separated "solid" acids is assured).

The employment of lead acetate in amount equal to the whole of the mixed acids seems, however, not to improve the separation and, indeed, definitely to impair it when the total content of saturated acids is low (*e.g.* below 25 per cent.).

It is essential that the temperature of the alcoholic solution should not fall below 15–16°C. during standing; exposure to a lower temperature leads to risk of still lower results than would otherwise be obtained. This may possibly have operated to some extent in some of the analyses marked *a* in Table III.

Our conclusions as regards the lead salt alcohol separation may be summarised as follows:

(i) The use of insufficient lead acetate for union with all the fatty acids present, as originally proposed by Twitchell, may lead, in certain cases, to results of doubtful analytical value, although the data obtained for the saturated acid content of mixtures of palmitic and stearic (or higher saturated) acids with oleic, linoleic, linolenic or palmitoleic acids are usually within 1–2 units of the true figure, and are sufficiently accurate for many purposes.

(ii) Employment of greater proportions (e.g. an amount equal to that of the total fatty acids taken) of lead acetate than those prescribed by Twitchell, leads to lower rather than to higher values for the fully-saturated acids, and cannot be recommended.

(iii) If the mixed fatty acids contain, in addition to the foregoing, more than 3 per cent. of myristic acid, the value obtained will commence to be appreciably lower than the true figure.

(iv) The saturated fatty acids of fats containing notable amounts of lauric or lower acids (such as the nut oils, butter-fats, etc.) cannot be satisfactorily analysed by the lead salt separation method.

(v) The saturated fatty acids of fats containing, as well as oleic acid, acids of the oleic series with 20 or more carbon atoms per molecule (fish oils, oils of the *Cruciferae* and *Umbelliferae*, etc.) cannot be satisfactorily analysed by the lead salt separation method.

(vi) Owing to small amounts of saturated acids passing into the "liquid" acids, even in the case of mixtures containing only palmitic and stearic acids, and still more so when myristic acid is also present, the iodine value of the "liquid" acids is somewhat lower than that due solely to the oleic, linoleic, etc., acids present. If the proportion of linoleic acid to oleic acid is not great, the error introduced by estimating this proportion from the observed iodine value of the "liquid" acids becomes disproportionately large. In any case, we do not recommend the estimation of the proportions of oleic and linoleic acids in a mixture of these two acids by means of the iodine value of the "liquid" acids from the lead salt separation.

(vii) The temperature of the alcoholic solution of lead salts must not be allowed to fall below 15–16° C.

(viii) Subject to the limitations set out above, there are many oils, vegetable and animal, which contain palmitic, stearic, oleic and linoleic acids with little or no myristic or other acid to which the lead salt separation, in its most effective form, can be usefully applied in the approximate determination (to within 1 to 2 units per cent.) of saturated higher fatty acids.

**SEPARATION OF SATURATED FATTY ACIDS AFTER REMOVAL OF UNSATURATED ACIDS BY OXIDATION.**—According to Bertram (*Z. deutsch. Oel und Fett-Ind.*, 1925,

45, 733) the alkaline solution (200 ml. from an original 5 grms. of fat) of soaps from the determination of saponification value and of unsaponifiable matter is cooled, 5 ml. of potassium hydroxide solution (50° Bé.) added, and a solution of potassium permanganate (30 grms.) in water (650 ml.) added whilst the mixture is kept below 25°. After thorough shaking, the mixture is left overnight, and then decolorised with warm concentrated bisulphite solution and dilute sulphuric acid. The oxidation is said to yield a mixture of higher saturated acids, together with dihydroxystearic acid, sativic and linusic acids, azelaic, nonoic, propionic and other lower acids from oleic, linoleic, etc., acids. Extraction with petroleum spirit (b.pt. 40–60° C.) is stated to remove the higher saturated acids and nonoic acid from the hydroxystearic acids and dibasic acids formed in the oxidation. The acids recovered from the petroleum spirit are dissolved in water (200 ml.) containing a little ammonia, 10 per cent. ammonium chloride solution (30 ml.) is added, and the solution boiled with excess of magnesium sulphate solution, which precipitates the higher fatty acid salts and leaves magnesium nonoate in solution. The filtered magnesium salts and also the combined filtrates and washings are separately treated with dilute sulphuric acid and the acids obtained are submitted to a repetition of the magnesium salt precipitation. The second precipitates of magnesium salts thus obtained are reconverted into fatty acids, which are extracted with petroleum spirit, and recovered from the latter solution and dried until constant weight is obtained.

This method, which has received favourable notice in many quarters, depends for its success on a number of factors which are considered below. Bertram (*Chem. Weekblad*, 1927, 24, 320) has admitted that it is not applicable to fats containing lauric or lower saturated acids or containing unsaturated acids (such as petroselinic,  $\Delta^6$ -oleic acid), which yield lauric or similar acids when disruptively oxidised; thus, to this extent, like the Twitchell process, it is restricted in its range of application. For synthetic mixtures of palmitic, stearic, arachidic, oleic and linoleic acids Bertram obtained results agreeing to within 0.1–0.7 unit with the calculated percentages of saturated acids present.

Clearly, for complete success, the following conditions are essential:

- (i) The oxidation of unsaturated acids must be complete;
- (ii) Dihydroxy- or other polyhydroxy-stearic acids produced by the oxidation must be completely insoluble in the petroleum spirit employed, whilst higher fatty acids must be completely taken up by this solvent;
- (iii) Lower fatty acids produced by the oxidation, and also dibasic acids (if not completely insoluble in the petroleum spirit) must be completely removed in the form of water-soluble magnesium salts.

In connection with the last point, we have found that magnesium nonoate and magnesium azelate are each sufficiently soluble to present no difficulty under the conditions given by Bertram for the magnesium salt separation, whilst the following data which we obtained with reference to magnesium laurate and myristate confirm

the statement of this author as to the limitations of the method as regards diminishing molecular weight of the higher saturated fatty acids:

Acid examined.	Grm.	Fatty acid in filtrates from Bertram magnesium salt separation.	
		Grm.	Percentage of acid taken.
Lauric .. ..	1.026	0.1425	13.9
Myristic .. ..	1.039	0.0190	1.8

The method is thus not suited for the determination of lauric acid, whilst myristic acid lies on the boundary line, and small proportions of this acid, when present with larger amounts of palmitic or stearic acid, or both, in a fat, can probably be dealt with fairly accurately in the magnesium salt separation, although losses of the order indicated must be expected to take place.

It seems to have been taken for granted hitherto that the oxidation of unsaturated acids by the Bertram process is complete, but we have found in all cases that the recovered "saturated" acids possess a small but definite iodine value. It is, therefore, desirable, as in the Twitchell method, to apply a correction based on this observed iodine value, with the result that, in many cases, the corrected figures obtained by the Bertram process are distinctly lower than either the uncorrected data or the values obtained by the fractionation method. Further, whilst it is logical to make this correction, the possibility must be borne in mind that in the case of the oxidation process it is possible for semi-oxidised products from oleic acid which do not show iodine absorption to be found in the final "saturated" acids; so that it is not certain that correction on the basis of observed iodine value will in this case allow for all the unsaturated acid derivatives which may be present in the final product.

The presence of semi-oxidised compounds of this type is probable, however, rather with permanganate-acetone oxidation (see below) than with the Bertram alkaline permanganate oxidation. In the latter case the first products of oxidation will be almost quantitatively dihydroxy- or polyhydroxy-stearic acids, and the important point here is the relative solubility of any of these acids and of the higher saturated fatty acids in the solvent employed for the final extraction of the latter.

We find that the petroleum spirit (b.pt. 40–60° C.) recommended by Bertram gives a satisfactory separation when no great amount of stearic or higher saturated acid is present, but, otherwise, it is difficult to ensure complete removal of stearic acid from the admixed dihydroxystearic acid by the use of this solvent, and in a number of experiments on beef tallow (*cf.* p. 356) we obtained low results. We studied the substitution of petroleum spirit (b.pt. 60–80° C.) and benzene for the lower-boiling petroleum spirit in this case, and found that, whilst the petroleum spirit of b.pt. 60–80° C. gave, on the whole, reasonably good results, benzene also dissolved some dihydroxy-stearic acid and gave a higher value for the saturated acids than the true one.

We came, consequently, to the conclusion that separation of higher saturated fatty acids from dihydroxystearic acid by selective solubility in an organic solvent is, perhaps, a weakness in the Bertram method, and we have endeavoured to use conditions of oxidation which will avoid, or reduce to a minimum, the formation of polyhydroxystearic acids. In the first place, we have carried out the oxidation with alkaline permanganate at a somewhat higher temperature ( $35\text{--}50^\circ\text{C}.$ ), when the dihydroxystearic salt initially produced is further broken down into suberic, octoic and oxalic acids (*cf.* Lapworth and Mottram, *J.C.S.*, 1925, 127, 1987); there seems no object in restricting the temperature to below  $25^\circ$ , since the saturated fatty acid soaps are not attacked under the conditions of oxidation. By this means the amount of dihydroxystearic acid in the final product is considerably reduced. Alternatively, the fat itself, instead of the mixed fatty acid sodium salts, is oxidised in solution in acetone with anhydrous potassium permanganate, in which case polyhydroxystearic acids are not produced, the initial product of oxidation being an oxygenated complex which breaks down into azelaic and nonoic acids; the final products of oxidation of the fat are recovered and completely hydrolysed, and the higher saturated fatty acids are then separated by the magnesium salt procedure as given by Bertram. Typical experimental results obtained during the course of this study will now be given in illustration of the foregoing statements.

(i) APPLICATION OF THE METHOD AS GIVEN BY BERTRAM.—The specimens of beef tallow, olive oil and cod-liver oil used in testing the Twitchell lead salt alcohol separation were again employed, and, except for variation in the organic solvent, as described in Table IV, the conditions prescribed by Bertram (*cf.* p. 360) were carefully followed throughout:

TABLE IV.

Fat.	Solvent employed.	Saturated acids isolated.			By fractionation analysis. Per Cent.
		Gross. Per Cent.	Iodine value.	Cor- rected. Per Cent.	
Beef tallow	Petroleum spirit, b.pt. $40\text{--}60^\circ\text{C}.$ *	52.2	1.4	51.4	54.2
	" " b.pt. $60\text{--}80^\circ\text{C}.$	53.5	3.3	51.5	"
	" " b.pt. "	56.6	3.2	54.6	"
	Benzene	60.6	1.8	59.4	"
Olive oil	Petroleum spirit, b.pt. $40\text{--}60^\circ\text{C}.$	13.3	3.8	12.7	12.6
	" " " "	13.4	2.8	13.0	"
Cod-liver oil	Petroleum spirit, b.pt. $40\text{--}60^\circ\text{C}.$	20.9	4.4	19.9	14.8
	" " " "	18.3	5.8	16.1	"

\* Other results were obtained in which the gross yield of saturated acids was obviously too low.

As in the case of the Twitchell method, the correction for iodine value cannot properly be applied to the figures for cod-liver oil because of the complex mixture of unsaturated acids therein present; the results are too high, possibly owing to

the presence of saturated acids produced by oxidation from some of the characteristic unsaturated acids in the oil, and the method seems as little suited as the Twitchell procedure for marine animal oils.

(ii) **BERTRAM'S METHOD, MODIFIED BY OXIDATION AT 35–50° C.**—In the experiments illustrated in Table V, the directions and quantities given by Bertram (*loc. cit.*) were followed, except that (a) the alkaline permanganate was added to the soap solution at about 35–40° C., and the temperature allowed to develop up to, but not beyond, 50° C.; (b) the solvent used was petroleum spirit (b.pt. 60–80° C.); (c) unsaponifiable matter was not removed prior to oxidation of the soap solutions.

TABLE V.

Fat.	Saturated acids isolated.			By fractionation analysis. Per Cent.
	Gross. Per Cent.	Iodine value.	Corrected. Per Cent.	
Beef tallow .. ..	55.1	2.8	53.4	54.2
Cottonseed oil	27.2	4.5	25.8	25.0
" "	28.7	8.8	25.9	
Borneo tallow	60.1	1.8	59.2	61.2
Cacao butter	59.4	2.0	58.1	58.8
Stillingia tallow	67.7	0.1	67.6	70.0*

\* The fractionation analysis indicated the presence of about 1.5 per cent. of lauric and 3 per cent. of myristic acid.

(iii) **OXIDATION OF AN ACETONE SOLUTION OF FAT WITH ANHYDROUS PERMANGANATE.**—The fat (5 grms.) was weighed out accurately into a 500 ml. (or 1000 ml.) round-bottomed flask and dissolved in acetone (250 ml. or 500 ml., according to the degree of unsaturation of the fat). Potassium permanganate (20 grms.) was added in small portions, with frequent shaking and gentle boiling under a reflux condenser, followed by more vigorous boiling for half-an-hour, when it was set aside overnight; next day, more potassium permanganate (5 to 30 grms.) was similarly added, the mixture again refluxed for half-an-hour and the acetone then removed by distillation. The residue was decolorised and acidified, and the organic products were extracted by ether. The residue from the washed ethereal extract was completely saponified with alcoholic potassium hydroxide and the soaps reconverted into acids, which were then submitted to the magnesium salt separation as described in the Bertram method.

The amounts of acetone and permanganate used depended on the unsaturation of the original fat, but the following general proportions may be recommended for a quantity of 5 grms. of fat:—

Original fat (5 grms.).	Acetone.	Permanganate.	
		1st stage.	2nd stage.
Iodine value 40 or below ( <i>e.g.</i> chocolate fats) ..	250 ml.	20 grms.	—
" " 40–55 ( <i>e.g.</i> tallows) .. ..	250 ml.	20 grms.	5–10 grms.
" " 80–110 ( <i>e.g.</i> olive oil, cottonseed oil)	500 ml.	20 grms.	15–30 grms.

The results obtained, calculated, as in the preceding cases, to the mixed fatty acids present in the fats oxidised, are given in Table VI.

TABLE VI.

Fat.	Saturated acids isolated.			By fractionation analysis. Per Cent.
	Gross. Per Cent.	Iodine value.	Corrected. Per Cent.	
Beef tallow .. .. .	{ 54.4 56.3	{ 1.8 2.2	{ 53.3 54.9	54.2
Olive oil .. .. .	13.9	3.7	13.3	12.6
Cottonseed oil .. .. .	27.1	3.1	26.2	25.0
Borneo tallow .. .. .	{ 62.7 66.3	{ 3.5 6.7	{ 60.3 61.4	61.2
Cacao butter .. .. .	59.3	1.4	58.4	58.8
Stillingia tallow .. .. .	70.2	2.8	68.0	70.0*

\* The fractionation analysis indicated the presence of about 1.5 per cent. of lauric and 3 per cent. of myristic acid.

Examination of Tables V and VI indicates that both methods give somewhat better figures than the Bertram method itself, although duplicate analyses still fail in some cases to give satisfactory agreement. With the Bertram oxidation carried out at 35–50° C., the experimental value for beef tallow is appreciably nearer to that determined by fractionation, and the other data in Table V, with the exception of those for Borneo tallow (the fatty acids of which contained 39 per cent. of stearic acid), are also within one unit of the percentage estimated by fractionation analysis.

The same order of agreement holds in Table VI (permanganate and acetone oxidation), and here the discrepancy in the case of Borneo tallow has disappeared; it is, however, noticeable that the more unsaturated oils now tend to give somewhat high figures for their saturated acid content, and this is probably to be attributed to the presence of traces of semi-oxidised unsaturated acids which cannot be corrected for by iodine value (*cf.* p. 362).

We have formed the following general conclusions regarding the oxidation processes for estimating saturated higher fatty acids:

(i) Although, in cases where palmitic and stearic acids are substantially the only saturated acids present, the (uncorrected) results obtained by following Bertram's method are usually close to the actual value, the saturated acids, as weighed, possess a small iodine value, and thus retain small proportions of unsaturated acids. When a correction is applied for the observed iodine value, the figures obtained for the saturated acids are frequently slightly low (1 to 2 units).

(ii) Alkaline oxidation of the soaps with permanganate at 35–50° C., or oxidation of the fats themselves in acetone with anhydrous permanganate, yields more consistent results than the low temperature alkaline oxidation proposed by Bertram.

(iii) When the saturated acids include none of lower molecular weight than palmitic (or myristic to the extent of not more than 5 per cent. of the total mixed fatty acids), the oxidation methods (ii) lead to results which should not be more than one unit lower than the true percentage, and which frequently lie well within



this limit. For such cases the modified methods suggested may be expected to yield somewhat more accurate results than the lead salt separation, even when the latter is carried out under the best conditions.

(iv) The oxidation methods are of no more use than the lead salt separations in the case of (a) fats containing notable amounts of lauric or lower acids (butters, nut oils, etc.) or (b) fats in which unsaturated acids other than oleic, linoleic and linolenic are present in quantity (*e.g.* marine animal oils, seed-fats of the *Cruciferae* or *Umbelliferae*).

It is obvious that, when oleic and linoleic acids only are known to be present, accurate estimation of saturated fatty acids permits the proportions of these unsaturated acids also to be deduced from the iodine value of the total mixed acids. In Table VII the data thus calculated from the results given in Table VI are compared with those obtained in the fractionation analyses.

TABLE VII.

Fat.	Iodine value.	Oxidation analysis.			Fractionation analysis.			
		Saturated acids.	Oleic acid.	Linoleic acid.	Saturated acids.	Oleic acid.	Linoleic acid.	Unsap. matter.
		Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Beef tallow ..	42.1	{ 53.3	44.1	2.4	54.2	42.6	3.0	0.2
		{ 54.9	40.9	4.0				
Olive oil ..	83.6	13.3	74.9	11.0	12.6	79.1	7.5	0.8
Cottonseed oil ..	104.7	26.2	24.8	48.3	25.0	29.3	44.9	0.8
Borneo tallow ..	32.3	{ 60.3	38.8	—	61.2	37.9	—	0.9
		{ 61.4	37.7	—				
Cacao butter ..	37.1	58.4	39.6	1.8	58.8	39.0	2.0	0.2

Naturally, any error in the saturated acids figure frequently leads to magnified differences in the deduced values for oleic and linoleic acids [the case of olive oil is exceptional, since the 0.8 per cent. of unsaponifiable matter present in the fat has an iodine value of about 195; allowing for this, however, the values for oleic and linoleic acids become, respectively, 76.7 and 9.2 per cent., this discrepancy being the result of the difference between the respective observed percentages (13.3 and 12.6) of saturated acids].

If the oxidation method for determining saturated fatty acids could be relied upon to within one unit per cent., the values for oleic and linoleic acids could be calculated within about  $\pm 2$  units per cent. In conjunction with the iso-oleic acid determination, as proposed by Cocks, Harding and Christian (p. 368), a fairly rapid assay of the saturated acids, oleic and iso-oleic acids, and linoleic acid present in hydrogenated fats, would thus become available.

SUMMARY.—1. The Twitchell lead salt and alcohol separation and the Bertram oxidation method for the rapid estimation of saturated fatty acids have been tested on a number of fats which had also been analysed by the fractionation method.

2. Neither method is suitable for fats which contain lauric or lower saturated acids or solid unsaturated acids containing more than 18 carbon atoms in the molecule. When, however, the mixed fatty acids are composed of not more than 3-5 per cent. of myristic acid with palmitic, stearic, oleic (iso-oleic), linoleic and/or linolenic acids (this covers a very large section of natural fats, including tallows, palm oils and many liquid vegetable oils) both methods are useful within the following limits:

3. The lead salt separation, as originally given by Twitchell, gives results not more than 1 to 2 units per cent. below the actual content of saturated fatty acids, providing that (a) the proportion of lead acetate to saturated fatty acids is maintained as directed by Twitchell, (b) the temperature of the alcohol solution is not allowed to fall below 15-16° C. during cooling.

4. The oxidation method, as described by Bertram, leads to the production of dihydroxystearic acid in quantity, and of saturated acids which still possess a definite iodine absorption. Modifications in the oxidation are suggested which reduce or eliminate the dihydroxystearic acid formation, but the saturated acids finally obtained are still accompanied by small amounts of unsaturated acids. A correction should be applied for the latter, on the assumption that the iodine value observed is due to oleic acid.

5. The original Bertram procedure gives results within 1 unit per cent. of the calculated value in some cases, but in others the values obtained may be lower; the modified procedures yield more consistent and higher values in the instances which have been examined.

6. For the rapid determination of saturated fatty acids, we are inclined to prefer the modified oxidation methods to the lead salt separation, although parallel determinations by both procedures in their improved forms are probably safest.

7. If the saturated acids can be determined within one unit per cent., and oleic (iso-oleic) and linoleic acids only are present, the amounts of each of the latter can be estimated at once to within about  $\pm 2$  units per cent. from the iodine value of the original mixed fatty acids, iso-oleic acids being determined, when present (hardened fats), by the method suggested by Cocks, Harding and Christian.

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## A New Method for the Determination of Solid Unsaturated Fatty Acids.

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(Read at the Meeting of the North of England Section, February 14, 1931.)

THE reliable determination of the solid saturated and solid unsaturated fatty acid contents of a fat or fat mixture is of great importance in a number of directions. Thus the knowledge of the proportions of these constituents is necessary in order to be able to follow the course of hydrogenation processes, and is of value in connection with (1) the consistence of margarine and pastry fats, and (2) the properties of soaps, as well as serving, in conjunction with other characteristics, as a means of recognising and approximately estimating partially hydrogenated oils in fat or fatty acid mixtures.

Until some six months ago, we, in common with very many chemists connected directly or indirectly with the oil and fat industries, employed the method given by Twitchell (*J. Ind. Eng. Chem.*, 1921, 13, 806) for determining the solid unsaturated fatty acid content of hydrogenated fats. Admittedly, Twitchell, in his original publication, gave only one example of the determination of solid unsaturated fatty acids, namely, in hardened cottonseed oil. Following the publication of his method, however, we examined it from the point of view of determining the solid unsaturated fatty acid content of different partially hydrogenated fats, and then came to the conclusion that, even if it were not entirely satisfactory, it undoubtedly represented an improvement on the lead salt and ether method of Gusserow and Varrentrapp, which had been largely employed prior to the publication of Twitchell's lead salt and alcohol method.

Some eighteen months ago our attention was directed by our colleague, Dr. A. Taffel, in London, to the discordant results obtained by different workers when using the Twitchell method for the determination of the total solid fatty acid content of partially hydrogenated whale oil. After a preliminary examination of the cause of the disagreement between determinations made at different times and in different laboratories, Dr. Taffel concluded that the method could be improved by increasing the proportion of lead acetate used for the formation of the lead salts, see p. 372.

While our investigation was in progress, Grossfeld (*Chem. Umschau*, 1930, 37, 3, 23; *Z. Unters. Lebens.*, 1930, 59, 237-258) published a method which was based

on the use of more dilute alcohol than that employed by Twitchell for precipitating, washing and recrystallising the lead salts. Grossfeld showed that by his modification he could detect the addition to cocoa butter of small amounts of partially hydrogenated arachis oil, but, as the result of a series of experiments with varying proportions of solid unsaturated acids, he concluded that in no case did his method return more than 63 per cent., and in many cases not more than 40 per cent. of the total solid unsaturated acids present. Further, when tested on mixtures containing a large proportion of oleic acid, his method became less reliable for the detection of small proportions of partially hydrogenated fats, on account of the precipitation of the lead salts of the liquid acids at the same time as those of the solid acids.

We had made tests on the use of aqueous alcohol on somewhat similar lines to those described by Grossfeld, and concluded that, while a much larger proportion of the total solid unsaturated acids was obtained than by the Twitchell process, there was still definite under-estimation of these acids, and at the same time there was a distinct risk of separation of liquid acids with the solid acids. We decided, therefore, that we required a method that would estimate a greater proportion of the actual solid unsaturated acids present, and would, at the same time, be free from the somewhat uncertain precipitation of liquid acids in conjunction with the solid acids.

At the commencement of this investigation, it was realised, in view of the results obtained by Moore (*J. Soc. Chem. Ind.*, 1919, 38, 320), Hilditch and Vidyarthi (*Proc. Roy. Soc., A*, 1929, 122, 552), and other workers, that a variety of iso-acids would be formed in the ordinary hydrogenation process, in addition to the preponderating elaidic acid. As these other acids possess melting points which may range from that of oleic acid or lower, up to that of elaidic acid or higher, some definition should be given for purposes of differentiating between solid and liquid, and for this reason it was decided that acids which were still solid at 20° C. should be classified as "solid acids."

**EXPERIMENTS EMPLOYING TWITCHELL'S METHOD.**—In the first place tests on the fatty acids from naturally occurring oils by Twitchell's method showed that similar results to those published by him were obtained, duplicate tests being within  $\pm 1.0$  unit per cent. The application of his method, however, to fatty acids from partially hydrogenated whale and soya-bean oils gave much less consistent results, duplicates varying, in the case of the former oil, by  $\pm 2$  units per cent. for the saturated acids and  $\pm 3$  units per cent. for the solid unsaturated acids.

In order to trace the reason for this variability in the results, and to see whether even the maximum figures represented the total quantity of solid acids present, six entirely comparable determinations were started side by side, and the filtrate and washings from the first precipitation were collected and placed on one side, and the filtrate and washings from the second precipitation were likewise kept, but not mixed with those from the first precipitation. Each of the alcoholic solutions was concentrated in two stages, at the end of each of which the liquid

was allowed to stand overnight at 15–20° C., and then any solid material was removed by filtration. The characteristics of the acids separated in this way are summarised in the following table:

TABLE I.

Fractions: Character of separated fatty acids	First Filtrate and Washings. Total amount of acids 37.9 per cent., expressed on original hydrogenated whale oil fatty acids.			Second Filtrate and Washings. Total amount of acids 14.5 per cent., expressed on original hydrogenated whale oil fatty acids.		
	1A. Solid	2A. Liquid	Residue A. Liquid	1B. Solid	2B. Semi- solid	Residue B. Liquid with some solid
Fatty acids expressed on original hydrogenated whale oil fatty acids, per cent.    ..    ..	6.25	2.1	29.05	6.2	4.3	4.0
Iodine value    ..    ..    ..	77.3	88.8	96.3	62.7	71.7	80.8
Melting point °C.    ..    ..	23–25	—	—	30.0	20–25	—

Re-conversion to lead salts of the above solid acids from fraction 1A, and re-crystallisation once from a proportionately reduced quantity of 92–93 per cent. (by weight) alcohol gave 5.9 per cent. of solid acids (m.pt. 26.9° C.) and 0.35 per cent. of acids which were practically completely liquid at 20° C., both percentages being expressed on the total weight of hydrogenated whale oil fatty acids taken initially. Similar treatment of fraction 1B gave 5.0 per cent. of solid acids (m.pt., 32° C.) and 1.2 per cent. of acids which appeared to be almost entirely solid at 20° C.

It will be seen from the iodine values and melting points of the solid fractions, in conjunction with the results obtained by reconvertng these fractions into lead salts and separating, that, while the solid acids are for the most part unsaturated, they evidently contain a small proportion of solid saturated acids, a fact which supports the findings of Hilditch and Priestman, see p. 360.

The above-described fractional separation not only demonstrates the large proportion of solid acids which are classified as "liquid" acids by the Twitchell process, but also serves to indicate in a roughly quantitative manner the minimum total amount of solid acids present in this sample of fatty acids from hydrogenated whale oil. Thus the total solid acids found by the Twitchell process, amounting on the average to 49.8 per cent., must be augmented by an amount of not less than 12.1 per cent., making a total solid fatty acid content of not less than 61.9 per cent. This last-mentioned figure may still be considered as a minimum, since, in the described method of separation, some solid acids will almost undoubtedly have remained dissolved as lead salts in the alcohol. This conclusion is confirmed by comparing the results obtained on this sample of whale oil when employing the new method to be described later (see p. 376).

Similar evidence of under-estimation by the Twitchell method was obtained by examining the filtrate and washings from the recrystallisation of the lead salts

of a series of Twitchell determinations on the fatty acids from partially hydrogenated soya-bean oil. From an initial separation of 5.3 per cent. solid acids, 3.5 per cent. solid acids (m.pt. 35° C.; iodine value, 80.0) were obtained on repeating the process. It was, therefore, clear that distinct amounts of solid unsaturated acids were escaping estimation by Twitchell's method.

In order to obtain further evidence regarding the quantitative character of this under-estimation of the solid unsaturated acids, the Twitchell method was applied to artificial mixtures of known composition. Representative solid unsaturated acids were prepared by partially hydrogenating the liquid acids from cottonseed oil so that practically no saturated acids were formed. The solid unsaturated acids (m.pt., 39° C.; iodine value, 83.6) which were separated from the remaining liquid acids by precipitation and two recrystallisations as lead salts from alcohol were mixed with varying proportions of cottonseed fatty acids, and tested by Twitchell's method with the following results:

TABLE II.

ARTIFICIAL MIXTURES OF COTTONSEED OIL FATTY ACIDS AND SOLID UNSATURATED ACIDS.

		Solid unsaturated acids, per cent.					
Added .. .. .	Nil	5.0	10.0	10.0	15.0	20.0	
Found by Twitchell method	0.1	0.9	0.5	3.9	6.3	8.8	

The solid unsaturated acids were thus very materially under-estimated, in some cases as much as 10 per cent. being present almost without detection, and in all cases the return being well under 50 per cent.

The under-estimation of this large proportion of the solid unsaturated acids by the Twitchell process was evidently due to the solubility of the lead salts in the alcohol. The influence of this factor, so far as the different lead salts are concerned, can be seen from the following table:

TABLE III.

SOLUBILITIES OF THE LEAD SALTS OF FATTY ACIDS IN 92-95 PER CENT. (BY WEIGHT) ALCOHOL EXPRESSED IN TERMS OF WEIGHT OF FATTY ACIDS IN 100 C.C. SOLUTION.

Fatty acid or fatty acid mixture.							Approx. solubility in grms. per 100 c.c.	Temp. °C.
Myristic .. .. .	..	..	..	..	..	..	0.0175*	12
Palmitic .. .. .	..	..	..	..	..	..	0.010*	16.5
Mixture of 83 per cent. myristic and 17 per cent. palmitic	..	..	..	..	..	..	0.03	17.5
Mixture of 60 per cent. palmitic and 40 per cent. solid unsaturated C <sub>18</sub> acid obtained from hydrogenated cottonseed oil by the Twitchell process—								
Palmitic constituent	..	..	..	..	..	..	0.02	17.5
Solid unsaturated constituent	..	..	..	..	..	..	0.06	
Total..	..	..	..	..	..	..	0.08	
Elaidic .. .. .	..	..	..	..	..	..	0.214†	20-22
Mixed iso-oleic acids from cottonseed oil	..	..	..	..	..	..	0.454	17.5
Mixed solid acids from hydrogenated whale oil fatty acids (iodine value 57.5) by the Twitchell process	..	..	..	..	..	..	0.20	17.5

\* Figures given by Twitchell, *loc. cit.*† Figure given by Grossfeld, *loc. cit.*

The above figures demonstrate the relative solubility of the different lead salts in alcohol and serve as an indication of the large losses of solid unsaturated acids which can take place.

### **Examination of Means for Improving the Accuracy of the Determination of the Solid Unsaturated Acids.**

With the object of finding a means of reducing the loss of solid acids which occurs in the Twitchell determination, the influence of the following factors, in addition to the effect of aqueous alcohol already referred to on p. 369, was examined: (1) The amount of lead acetate for precipitation; (2) The use of lead acetate solution for washing and recrystallisation; (3) Conditions of cooling; (4) Single crystallisation of lead salts; (5) Use of solvents other than ethyl alcohol.

1. **THE EFFECT OF THE AMOUNT OF LEAD ACETATE EMPLOYED IN THE PRECIPITATION.**—As already mentioned on p. 368, an improved yield of solid acids could be obtained in the case of partially hydrogenated whale oil by increasing the amount of lead acetate used for the precipitation. The extent of the improvement, however, even when the amount of lead acetate used corresponded to 140 per cent. of that required theoretically by all the fatty acids present, was only small, and amounted to an additional 3 to 4 per cent. of solid acids. Generally speaking, the use of this equivalent of lead acetate was highly desirable for mixtures containing a preponderance of solid acids, but with those containing less than 25 per cent. of solid acids it was most important to use about 100 per cent. excess of lead acetate above that required for the solid acids only, since, if this amount were exceeded, lead salts of the liquid acids were precipitated in quantity.

(2) **THE EFFECT OF THE USE OF LEAD ACETATE SOLUTION FOR WASHING AND RECRYSTALLISATION.**—It was found experimentally that a concentration of 1.5 per cent. to 2.0 per cent. of lead acetate,  $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$ , represented the optimum concentration for depressing the solubility of the more soluble lead salts of the solid fatty acids. The results obtained, however, even when a quantity equivalent to 140 per cent. of that theoretically required by the total fatty acids was used initially in the precipitation, were only slightly more satisfactory in respect of the return of solid acids, and still showed that very appreciable losses of these acids occurred.

(3) **CONDITIONS OF COOLING.**—By allowing the crystallisations of the lead salts in the first and second precipitations to continue for varying lengths of time it was shown that gradual precipitation took place, but even after five days at 15–20° C., the yields obtained were still distinctly low.

Variations in the temperature of cooling showed that it was unsafe, at any rate with certain mixed fatty acids, to cool to 0° C. on account of the precipitation of the lead salts of liquid acids. Generally speaking, it appeared that a cooling temperature of 15–20° C., which should be reached gradually, was the most satisfactory, and as this coincided with Twitchell's conditions, it was concluded that no improvement in the process could be effected by changing the temperature of cooling.

(4) **SINGLE CRYSTALLISATION OF LEAD SALTS.**—The possibility of being able to estimate the solid fatty acids without having to recrystallise the lead salts was investigated fully, in spite of the fact that Twitchell (*loc. cit.*) had indicated in his original paper that no amount of washing without recrystallisation would render the lead salts of the solid fatty acids sufficiently free from liquid acids. It was found that by single crystallisation followed by very thorough washing with alcohol, the amount of retained liquid acids could in many cases be reduced to a reasonable limit, but in others this seemed impossible. However, the volume of alcohol necessary was so large as to cause distinct loss of the lead salts of the solid unsaturated acids. This loss of solid unsaturated acids by washing, coupled with the necessity for such thorough washing, prompted the search for a more suitable solvent than alcohol.

(5) **THE USE OF SOLVENTS OTHER THAN ALCOHOL.**—The possibility of using solvents other than alcohol for effecting the separation between solid and liquid acids has been considered and applied by various workers in the past, but, for one reason or another, the processes have not proved entirely satisfactory. Attempts were, therefore, made to see if some improved method could be devised.

In the first place, experiments on the solubility of the lead salts prepared from partially hydrogenated whale oil fatty acids by three crystallisations from alcohol, showed the following results:

TABLE IV.

Solvent.	Fatty acids in solution per 100 c.c. at 15–17° C.	
	Grm.	
Ethyl alcohol (92–95 per cent. by weight)	..	0.035
Methylated ether .. .. .	.. ..	0.007
Petroleum spirit, b.pt. 40–60° C. .. ..	.. ..	0.005
Acetone .. .. .	.. ..	0.014

As petroleum spirit seemed to be the solvent which dissolved the lead salts of the solid acids to the least extent, and as it readily dissolved the lead salts of the liquid acids, a further series of comparisons on the solubility of the lead salts of the solid acids from hardened whale oil in 92–95 per cent. (by weight) alcohol and in petroleum spirit was carried out on the lead salts which had only been precipitated once and washed with the minimum quantity of 92–95 per cent. (by weight) alcohol. The results of successive extraction treatments by the two solvents on such lead salts are shown in the following table:

TABLE V.

				Approximate solubility of lead salts expressed in grms. of fatty acids in solution per 100 c.c. of solvent at 15–17° C.	
				Alcohol (92–95 per cent. by weight).	Petroleum spirit (b.pt. 40–60° C.).
First extraction	..	..	..	0.560	0.180
Second	..	..	..	0.210	0.021
Third	..	..	..	0.137	0.022
Fourth	..	..	..	0.094	0.012
Fifth	..	..	..	0.074	0.010



The above figures, apart from the first extraction, in which probably both solvents were removing a certain proportion of retained liquid acids, show that the solubility of the lead salts is of the order of seven to ten times greater in the 92-95 per cent. (by weight) alcohol than in the petroleum spirit.

The possibility of using petroleum spirit as the sole solvent for the determination was considered, but it soon became clear that some other solvent must be used, at any rate, for the formation of the lead salts. Methylated ether, on account of the fact that its power for dissolving the lead salts of the solid acids is only slightly greater than that of petroleum spirit, was also tested without the use of any other solvent for the formation of the lead salts, but the results did not prove satisfactory. The use of petroleum spirit was, therefore, tried, after forming the lead salts in alcoholic solution, as in this way the lead salts could be obtained in a readily-washable, crystalline condition, in contrast with their sticky and unmanageable character when prepared by double decomposition from aqueous solution (*cf.* Gusserow-Varrentrapp). This formation of the lead salts in alcoholic solution, and their subsequent treatment with petroleum spirit, forms the basis of the new method of determining the solid acid content of mixtures, and the experimental evidence upon which the new method was devised will now be described.

**BASIS OF THE NEW METHOD OF DETERMINATION.**—The advantages to be anticipated from a method involving the formation of the lead salts in alcohol, followed by thorough washing with petroleum spirit, were that, by controlling the quantity of alcohol used for the precipitation in relation to the amount of acids employed in the test, the loss of solid unsaturated acids through the solubility of their lead salts in alcohol should be reduced to a minimum, and washing of the lead salts with petroleum spirit should remove the liquid acids and their lead salts without dissolving out more than mere traces of the lead salts of the solid saturated and unsaturated acids. Experiments showed, however, that the alcohol used for the precipitation of the lead salts must not contain too great a concentration of liquid acids, otherwise the lead salts of these acids were precipitated in large quantities and became difficult to remove even with petroleum spirit. Furthermore, there was evidence that when the co-precipitation from alcohol of the lead salts of the solid and liquid acids took place, appreciable quantities of mixed lead salts of solid and liquid acids were formed, with the result that the efficiency of the petroleum spirit washing treatment was considerably impaired.

Preliminary experiments indicated that on naturally occurring unhardened fats, the new procedure involving the precipitation of the lead salts from alcoholic solution and washing the precipitate with petroleum spirit gave an iodine value of the separated fatty acids somewhat higher than that generally found by Twitchell's method. Thus, with the latter, the apparent amounts of solid unsaturated acids ranged from 0.1 to 0.3 in natural oils and fats (excluding tallow), whilst with the new method the amounts ranged from 1.9 to 3.4. On the other hand, however, with samples of partially hydrogenated whale, olive, cotton and

soya-bean oils, the amounts of solid unsaturated acids were from 1.5 to three times greater by the new method than by Twitchell's method.

In order to test the value of the new procedure in respect of its efficiency for estimating solid unsaturated acids, determinations were carried out on artificial mixtures containing known amounts of these acids. While the results were much closer to the actual than by the Twitchell method, there still appeared to be an appreciable under-estimation of the solid unsaturated acids. Stagewise concentration of the alcoholic filtrate, followed by overnight cooling periods at 15–20° C., did not give any further deposit of the solid acids, but examination of the material removed by the petroleum spirit washing treatment showed that a small quantity of solid acids had been dissolved. These solid acids could be recovered by removing the petroleum spirit and dissolving the residue in a small proportion of boiling alcohol containing 0.5 per cent. acetic acid, from which solution the lead salts of the solid acids precipitated almost immediately on cooling. Typical examples of the amounts, melting points and iodine value of the solid acids which were recovered in this way from tests which had been carried out by the new procedure involving washing with petroleum spirit, are given in the following table:

TABLE VI.

From:	Fatty acids. Iodine value.	Solid acids dissolved from lead salts by petroleum spirit.		
		Expressed on total fatty acids.	M.pt. °C.	Iodine value.
		Per Cent.		
Partially hydrogenated whale oil ..	57.5	6.0	18–20	89.0
„ „ cottonseed oil ..	74.7	7.4	29–30	81.0
„ „ olive oil ..	35.7	3.0	27	73.9
„ „ soya-bean oil ..	86.6	7.2	25	84.0

The cause for this removal of solid fatty acids by the petroleum spirit washing appears to be either the presence of certain particular solid unsaturated acids or the formation of mixed lead salts. The latter seems to be the more probable explanation, as the amount of solid acids which pass into solution in the petroleum spirit varies not only with the particular sample of partially hydrogenated fat, but also with the proportion of fatty acids and alcohol in the first precipitation. It, therefore, became necessary to include this additional procedure in the method, so as to be sure that appreciable proportions of solid acids were not escaping estimation.

A feature of great importance in this new method is the avoidance, as far as possible, of the formation of insoluble lead oleate, and, therefore, the proportions of fatty acids, lead acetate and alcohol must be rigidly adhered to, and care must be taken to prevent the temperature falling below 15° C. during the precipitation of the lead salts from alcohol.

**DESCRIPTION OF THE NEW METHOD OF SOLID FATTY ACID DETERMINATION.—**

In all cases a quantity of 3.5 grms. of the freshly prepared fatty acids\* is dissolved in 50 c.c. of 92–93 per cent. (by weight) alcohol and (except with liquid oils or mixtures containing less than 25 per cent. of solid acids), 3.45 grms. of lead acetate,  $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$ , is dissolved in 50 c.c. of similar alcohol. (With mixtures containing less than 25 per cent. of solid acids the same weight of freshly-prepared fatty acids is taken, but only 1 gm. of the lead acetate is dissolved in the alcohol.) Both solutions are heated to boiling point, and that containing the lead acetate is poured into the one containing the fatty acids. After mixing and again heating to boiling, the solution is allowed to cool slowly and evenly and to stand overnight† at 15° C. to 20° C., which limiting temperatures must be adhered to strictly (being checked by a maximum-minimum thermometer).

The next morning the mixture is stirred and filtered from the alcohol as completely as possible on a 10 cm. Buchner funnel, which is then transferred and fixed to a clean filter flask. The lead salts on the Buchner funnel, and any remaining in the precipitating vessel, are washed with 100 c.c. of petroleum spirit (b.pt. 40–60° C.), using amounts of 20 c.c. at a time, and the washings are then distilled from a water-bath to remove the petroleum spirit completely. The residue is dissolved by boiling under a reflux condenser with 20‡ c.c. of 92–93 per cent. (by wt.) alcohol containing 1 drop (0.05 gm.) of glacial acetic acid, added from a pipette. The solution is allowed to crystallise at 15–20° C. for 3 hours. The lead salts of the solid acids which precipitate readily under these conditions from the alcohol are filtered and washed with 20 c.c. of cold 92–93 per cent. alcohol, and the solids remaining behind on the filter paper are worked up with the main bulk of the lead salts of the solid acids left in the Buchner funnel after washing with petroleum spirit, the method being to decompose the lead salts with dilute nitric acid in the presence of methylated ether, washing the latter free from mineral acid and removing the solvent.

The total solid fatty acids are weighed and the whole, or a portion, is used for iodine value determination, from which the amounts of solid saturated and solid unsaturated acids can be calculated on the basis that the latter will have a mean iodine value of 90, unless evidence is available to show that some other iodine value should be adopted, *e.g.* erucic acid from rape oil, iodine value 75.5.

\* Consideration has been given to the possibility of starting the estimation from glycerides. It has been shown that neutralisation of the alkali used for alcoholic saponification cannot be effected with acetic acid without giving rise to low results. Sulphuric and oxalic acids, on account of the relative insolubility of their sodium salts in alcohol, are much superior for the purpose, but, as yet, it has not been established that the results obtained are so reliable as those in which the freshly prepared fatty acids are employed.

† In the event of a result being required as quickly as possible, the cooling period may be reduced to 2½ hours, half-an-hour being taken for the mixture to cool in air on an asbestos mat, and two hours subsequent cooling in water at 15–20° C. If this procedure is adopted the yield of solid acids will generally be some 2 per cent. low in comparison with the method of cooling overnight.

‡ In exceptional cases of very slightly hardened fats containing high proportions of liquid acids the lead salts may separate in a liquid or semi-liquid condition when only 20 c.c. of alcohol are used. In such instances the quantity of alcohol, containing the same proportion of acetic acid, must be increased until no separation of the lead salts occurs in the liquid condition.

COMPARISON BETWEEN THE NEW METHOD AND TWITCHELL'S METHOD. The figures in the following table represent a summary of the comparisons that have been carried out between the new method and Twitchell's method for the determination of the solid saturated and solid unsaturated fatty acid contents in naturally occurring and partially hydrogenated oils and fats.

TABLE VII.

Fatty acids from naturally occurring oils.	Iodine value of fatty acids.	Solid saturated acids.		Solid unsaturated acids.	
		Twitchell's method.	New method.	Twitchell's method.	New method.
		Per Cent.	Per Cent.	Per Cent.	Per Cent.
Olive .. .. .	85.6	11.0*	12.3	0.3	1.9
Cottonseed	107.0	23.6	24.2	0.1	1.9
Soya-bean	138.5	11.7	11.9	0.1	2.1
Palm ..	53.7	46.9	47.4	0.3	3.4
Tallow	46.5	48.7	48.8	1.7	5.6
Coconut	9.1	47.5	63.4	nil	1.3
Rape ..	105.8	4.4	1.2	47.5	52.2
Fatty acids from partially hydrogenated oils.					
Whale .. .. .	57.5	37.7	40.0	12.1	27.0
Whale ..	72.4	26.6	28.7	18.0	33.5
Whale ..	37.2	52.1	57.4	9.8	16.8
Cottonseed	74.7	26.6	29.6	15.2	34.4
Cottonseed	74.3	30.0	30.1	15.3	25.6
Cottonseed	80.1	26.5	26.9	23.1	32.3
Olive ..	80.0	15.1	14.9	5.9	20.8
Olive ..	73.5	21.5	21.8	12.6	22.6
Olive ..	35.7	62.3	61.2	11.5	26.2
Soya-bean	86.6	17.6	18.4	33.1	38.6
Soya-bean	59.6	34.3	34.4	32.0	39.5
Arachis	78.4	16.5	19.9	26.9	53.0
Arachis	62.4	29.7	32.2	30.3	45.4

\* Using half the concentration recommended by Twitchell.

The above series of comparisons shows that, as a means of determining solid saturated acids in naturally occurring oils, containing no acids of lower molecular weight than myristic, the new method is as satisfactory as Twitchell's method. From the coconut oil result it will be seen that the method returns more lauric acid than the Twitchell method, but even so, its estimation of this acid is not complete. The most important feature, however, of the new method is the way in which it enables the solid unsaturated acids to be estimated to a much greater degree of accuracy than is possible by Twitchell's method.

From the evidence submitted it will be realised that the new method will, so far as solid unsaturated acids are concerned, involve two small balancing errors, the first depending on the solubility of the lead salts of the solid unsaturated acids in the alcohol used for the precipitation, and the second on the fact that the solid saturated acids from naturally occurring fats appear to be contaminated with a small amount of liquid acids (unless such amounts of solid unsaturated acids are naturally present). The significance of the former loss is not great in mixtures

containing appreciable proportions of liquid acids, as these appear to exert a marked depressing influence on the solubility of the lead salts of the solid unsaturated acids in the alcohol. The small amount of the loss in this direction is shown by the difficulty in obtaining any solid acids by concentrating and cooling the alcoholic liquor after removal of the insoluble lead salts. In practice, in all but extreme cases with either very small amounts of liquid unsaturated or very small amounts of solid unsaturated acids, the two errors tend to balance one another.

In order to obtain information regarding the degree of accuracy that may be expected from the new method, firstly, artificial mixtures of cottonseed oil fatty acids and solid unsaturated fatty acids isolated in the manner referred to previously (p. 371); and, secondly, artificial mixtures of palm oil fatty acids and solid unsaturated acids prepared from the liquid acids of soya-bean oil by partial hydrogenation and then separation under conditions similar to those used in the new method of determining solid unsaturated acids, were tested with the following results:

TABLE VIII.

		Artificial mixtures made to the following composition.		Composition found by new method.	
		Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.	Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.
Cottonseed fatty acids ..		—	—	21.2	3.3
Mixtures .. ..	{	22.6	5.0	22.3	8.7
		21.3	12.1	21.6	15.1
		19.4	23.2	20.1	21.7
		16.4	40.0	17.1	36.8

For comparison with the Twitchell method see Table II. on p. 371.

TABLE IX.

		Artificial mixtures made to the following composition.		Composition found experimentally.			
				Twitchell's method.		New method.	
		Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.	Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.	Solid saturated acid. Per Cent.	Solid unsaturated acid. Per Cent.
Palm-oil fatty acids .. ..		—	—	44.9	0.3	45.3	3.8
Solid unsaturated acids (iodine value 82) from soya-bean oil ..		—	—	7.0	61.9	6.1	70.8
Mixtures .. ..	{	44.7	2.0	44.2	0.7	45.1	5.6
		43.6	5.0	42.4	1.4	43.6	6.5
		38.2	20.0	37.0	7.6	36.3	17.4

From each of the series of tests it will be seen that with the smallest additions of solid unsaturated acids there is an apparent over-estimation, due to the fact that

the original mixtures to which no solid unsaturated acids had been added show some 3.5 per cent. of apparent solid unsaturated acids. These results represent extreme cases in one direction. In the other direction the extreme case is covered by the tests on the solid unsaturated acids from the soya-bean oil. The fact that the new method returns only 9 per cent. more solid unsaturated acid than does the Twitchell method, and shows an under-estimation of some 23 per cent., is due largely to the absence of liquid acids, which normally depress to a marked degree the solubility of the lead salts of the solid unsaturated acids in alcohol. From data collected on these solid unsaturated acids, prepared from the liquid acids of soya-bean oil by partial hydrogenation, it is considered that some 3-4 per cent. consisted of oxidised acids and some 3-4 per cent. of liquid unsaturated acids, so that the loss of solid unsaturated acids by the new method is probably about 16 per cent., in comparison with 25 per cent. by the Twitchell method. The accuracy of the new method is such that, for determining intermediate amounts of solid unsaturated acids of the order usually occurring in partially hydrogenated fats, either alone, or as an appreciable proportion of fat mixtures, it is considered to give results which are at least within 3.0 units per cent. of the actual solid unsaturated acid content (m.pt. 20° C. or above). In contrast with this, the Twitchell method cannot be relied on to anything like the same degree of accuracy, as the under-estimation is generally of the order of 10 units per cent., and in certain instances may be much greater.

The new method, when applied to rosin alone, showed a return of 56-58 per cent. of "solid" acids. In admixture with fatty acids, however, this figure was not proportionately maintained, a fact which may be connected with the unsaponifiable matter. In general, therefore, the degree of accuracy of the method is impaired somewhat by rosin, the presence and amount of which can readily be determined.

THE SEPARATION OF SOLID FROM LIQUID ACIDS BY MEANS OF SALTS OTHER THAN THOSE OF LEAD.—During the present investigation there have been several indications that lead salts were not ideal for the separation of solid and liquid acids by means of a fractional precipitation or crystallisation method, on account of the possibility of the formation of mixed lead salts containing both solid and liquid acids. Although it has been shown that, by adhering to definite concentrations of acids and precipitating salt, this mixed salt formation can be reduced to a minimum, it was thought that some univalent metal salt might be preferable. Thus, methods based on the use of silver and thallium salts for effecting the separation were subjected to a preliminary examination, but the results obtained did not seem to warrant their use in preference to the lead salts.

SUMMARY.—(1) The marked extent to which Twitchell's method under-estimates the solid unsaturated acids in partially hydrogenated fats has been shown, firstly, by the separation of solid acids which escape determination by that method; and secondly, by carrying out tests on artificial mixtures of known composition.

(2) The marked degree of under-estimation of the solid unsaturated acids by the Twitchell method is almost entirely due to the solubility of the lead salts of these acids in the 95 per cent. (by weight) alcohol employed for precipitation, crystallisation and washing.

(3) Certain modifications in Twitchell's method have been found to improve the accuracy to only a small extent. However, by means of the new method, involving the use of petroleum spirit (b.pt., 40–60° C.), in conjunction with the formation of the lead salts in alcohol, as described on pp. 374 and 376, a much more accurate determination is possible in a somewhat shorter time.

(4) Thus: (a) in mixtures containing solid unsaturated acids Twitchell's method only slightly under-estimates the solid saturated acids (*cf.* Hilditch and Priestman (*loc. cit.*) for naturally occurring fats), and materially under-estimates the solid unsaturated acids (Table I, p. 370, Table II, p. 371, Table IX, p. 378), generally to the extent of some 10 units per cent., and sometimes even more; (b) the new method, however, gives a rather more accurate return of solid saturated acids, and a definitely more accurate estimation of the solid unsaturated acids, which are determined, in all but extreme cases not likely to be met with in practice, to within 3 units per cent. of the actual.

In conclusion, we wish to thank Messrs. Lever Bros. Ltd. for permission to publish the results of this investigation.

RESEARCH LABORATORIES,  
PORT SUNLIGHT.

## The Rising of Fat in Milk.

BY GUNNER JØRGENSEN.

As a supplement to the experiments of Stock (ANALYST, 1930, 55, 535), I should like to give the results of some tests, just completed, which were made in order to answer a question, raised by the police, as to the probable fat content of a sample drawn from the bottom tap of a churn containing milk which had been standing for about one hour.

From a general consideration of the question it appears that the depth of the layer of the very poor milk formed after a certain time at the bottom does not depend particularly on the depth of milk in the churn; so that, whether this is, for instance, 20 or 100 cm., the rise of fat in the lowest mm. or cm. of the milk will take place at virtually the same rate for the same milk under similar conditions. On the other hand, the distance of the bottom tap from the base of the churn has a bearing upon the quantity of fat in a small quantity of the milk drawn, as has also the velocity with which the milk is drawn. By slow drawing, the milk from the layer in front of the tap will be able to run out without getting mixed with the surrounding

layers; but, if the withdrawal takes place quickly, some milk from the surrounding layers, particularly the upper ones, will be drawn down to the tap and cause too high a fat-content in the sample. Furthermore, the proportions between the diameter of the churn and the quantity of milk drawn, will—so long as only the very lowest layers of milk have given up a considerable part of their fat—have an influence upon the amount of fat in the sample drawn.

In conformity with these considerations, I have made the tests by using two glass-vessels of different size and form. In the narrower of these vessels 3 decilitres of milk reached a height of 35 cm., whilst 15 decilitres of milk in the wider vessel reached a height of 23 cm.

The sides of the lower parts of these vessels were vertical, and were fitted with glass tubes with rubber tubing and pinch-cocks. After the milk had stood for a recorded time, 15 ml. were drawn off and tested. This volume represented, for the narrow glass, a height of about 3 cm.; and, for the wider glass, about 0.8 cm., measured from the top of the glass-tube, the volume of which was only 1.5 ml. For this reason the quantity of fat separated from this volume of milk has very little influence upon the fat content of the milk above.

The milk used, containing 3.75 per cent. of fat, had been heated at 50° C. for a short time and then re-cooled.

The samples drawn contained:

After standing for	The narrow glass. Per Cent.	The wide glass. Per Cent.
1 hour	2.90	1.35
2    "	2.60	1.10
24   "	0.25	0.15

From the upper layers in the vessel with 15 decilitres of milk, samples of different amounts were drawn through the glass tube after standing for 24 hours.

The distances of the samples from the bottom of the vessel were:

Cm.	Fat. Per Cent.
15.0-15.4	1.45
6.0-15.0	1.45
2.0- 6.0	0.95
0.8- 2.0	0.35
0 - 0.8	0.15

A comparison of these results with Tables VI-IX of Stock shows, that, after one hour, Stock found a considerably lower decrease of fat in the samples taken from the bottom tap than after two hours, whilst I find the greatest decrease after the first hour. There is no doubt that this discrepancy would have disappeared if Stock had placed the tap in the middle of the bottom of the churn and not one-and-three-quarter inches (=4.5 cm.) above the bottom. Thereby he would undoubtedly have found a greater decrease for the contents of fat in the lower part of the milk than he did.



Stock's tables also show a very considerable difference for the velocity with which the fat rises in his four samples of milk.

The following table gives the percentage alterations in contents of fat:

	Stock's test.				Jørgensen's tests.		
	During 1 hour.		During 2 hours.		Glass-vessel.	Decrease.	
	Decrease, bottom-tap.	Increase, top-milk.	Decrease, bottom-tap.	Increase, top-milk.		During 1 hour.	During 2 hours.
VIII	nil	0.05	0.05	0.35			
VII	0.02	0.15	0.12	0.57	Narrow	0.85	1.15
VI	0.22		0.97	2.15	Wide	2.40	2.65
IX	0.21	1.33	0.77	5.23			

Finally, a few words about the alteration in the contents of the solids-not-fat mentioned by Stock. In the fat-free "top milk" in his Table IX the contents of solids are calculated as follows:

	Per Cent.	Fat in the corresponding milk.
		Per Cent.
Immediately .. .. .	9.39	3.32
After standing for 20 minutes	9.28	3.42
40 "	9.31	3.62
1 hour	9.36	4.65
1 " 20 minutes	9.42	5.85
1 " 40	9.41	7.57
2 hours	9.68	8.55
2 " 20 minutes	9.67	10.05
2 " 40 "	9.60	10.95

According to this there is no visible increase of solids-not-fat for an augmentation of fat from 3.22 to 7.57 per cent., but for the samples with higher percentages of fat the increase suddenly becomes fairly constant.

On studying the figures in Tables VI-IX, recorded immediately after the placing of the milk in the churn, although these figures for each table should be the same, yet one observes a difference between the percentages of fat, up to 0.10 (Table IX), and between the total solids, up to 0.27 (Table VII). We may then take it for granted, that there may be differences in the figures of solids-not-fat up to 0.37 per cent., so that, in my opinion, there is, in the results given by Stock, nothing to show that an accumulation of fat in the upper layers of milk does involve an increase of solids-not-fat.

I do not, however, regard it as out of the question for such an alteration to take place, because the globules of fat are believed to be inclosed in a thin film of albumin. On the other hand, the milk fat will be oxidised by drying in the air, and, therefore, an increase of weight will take place, but this increase may be considered of about the same magnitude for the fat-containing solids and for the fat, and, consequently, the weight of solids-not-fat should be about the same for a rich and for a poor milk.

## The Examination of Eggs suspected of being "Preserved."

By JOHN RALPH NICHOLLS, B.Sc., F.I.C.

SECTION 3 of the Agricultural Produce (Grading and Marking) Act, 1928, makes it unlawful "to sell or expose for sale any egg which has been subjected to any process of preservation unless the egg is marked in the prescribed manner." The Minister of Agriculture and Fisheries has power to "exempt from the operation of this section eggs preserved by any process with respect to which he is satisfied that the marking of eggs preserved by that process cannot be enforced." Difficulty of enforcement occurs where it is not possible to ascertain by examination whether eggs have, in fact, been preserved by any process; and the Minister of Agriculture issued an Order, dated 16th October, 1928, exempting eggs preserved by "cold storage and by chemical storage." The latter term is used in a limited sense, and is defined in the Act as "storage for the purpose of preserving eggs by any process which does not alter the composition of the shells, including storage in any gas, vapour or gaseous mixture."

The effect of this Order is to limit the present operation of Section 3 to eggs preserved by processes which alter the composition of the shell, and, in accordance with the Agricultural Produce (Grading and Marking) (Eggs) Regulations, 1928, Statutory Rules and Orders No. 984, such eggs must be marked "PRESERVED."

For the examination of samples taken under this Section it is necessary to have means for detecting when the shell's composition has been altered. The present paper gives the results of tests carried out with this object in view.

**METHODS OF PRESERVATION.**—The methods commonly in use, other than those included in the above-mentioned exemption, may be divided into three classes:—

- (1) Treatment with oil or similar fluid.
- (2) Immersion in sodium silicate solution.
- (3) Immersion in lime water.

Eggs preserved by these processes will be referred to as oiled, silicated, and limed eggs, respectively.

(1) *Preservation with Oil.*—In this process the eggs are immersed in oil or other similar fluid, so that on removal each egg is covered with a thin film. Among the substances which are stated to be suitable for this purpose are the following:—Mineral oil (with or without waxes, gums and resins), molten paraffin wax, poppy oil, linseed oil, tung oil, drying oil with or without resins and thinners. Patents have been granted for such protective coatings as camphor, celluloid and amyl acetate after a first coating of gelatin, collodion after a first fatty coating, and synthetic resins, but no eggs covered with such protective coatings have been encountered.

In some cases the eggs are dipped in the cold liquid; in others, hot oils at 210–260° F. are used, the eggs being immersed for not more than a few seconds. One preserving medium for home use is sold as a grease which is to be placed in the palm of the hand and in which the egg is rolled until completely covered.

In connection with this process of preservation it is to be noted that eggs intended for cold storage are sometimes "processed" or "sterilised" by dipping them in hot oil, in order to prevent evaporation of water from the contents of the eggs during the time they are in cold store. In such cases the process is not primarily designed to replace cold storage, but rather to prevent deterioration in the quality of the eggs. Whether such eggs are entitled to be sold without being marked "PRESERVED" is doubtful.

(2) *Preservation in Sodium Silicate Solution.*—The eggs are completely immersed in a 3 to 10 per cent. solution of sodium silicate (water-glass), and are usually allowed to remain therein until required. On removal, the eggs are generally rinsed before sale.

(3) *Preservation in Lime Water.*—This is similar to (2), the solution of sodium silicate being replaced by saturated lime water. An "ice" of calcium carbonate forms on the surface of the bath and retards the diminution of strength of the alkaline liquid by the air.

**MECHANISM OF PRESERVATION.**—A freshly-laid egg is warm and the contents completely fill the shell. On cooling, slight contraction occurs and an air space forms between the egg membrane and shell membrane. This air has been sucked through the pores of the shell. When exposed to the air the shell contents lose water by diffusion and evaporation through the shell, the air space gradually increasing in volume and being, in some measure, an indication of the age of the egg. Bacteria can also gain access to the contents through the shell, and the longer the exposure the greater will be the contamination from this source. Since an egg submitted to preservation processes as soon as it has cooled (which is the most suitable time) can be retained for considerable periods without decomposition, whilst stale eggs similarly treated continue to deteriorate, it is probable that bacterial contamination is the primary cause of decomposition. Preservation with oil closes the pores of the shell with a film of oil which prevents ingress of bacteria. Silicate or lime solutions, which are both alkaline, may derive their preserving properties from their bactericidal action. On the other hand, they might mechanically close the pores of the shell. To investigate this point, tests have been carried out on the porosity of the shell of unpreserved and preserved eggs, and it has been shown that both silicate and lime solutions close the pores. This explains the well-known household experience that preserved eggs generally crack on being boiled. Such alteration in the porosity of the shell is characteristic of the three types of preservation under consideration. It is readily demonstrable and may be regarded as proof of preservation. Tests are described later for the characterisation of the method of preservation.

**POROSITY OF THE SHELL.**—Attempts were first made to obtain a quantitative measure of the degree of porosity by finding the quantity of water capable of being absorbed by the shell. Weighed pieces of shell were placed in a vessel which was then evacuated by means of a mercury pump. Water was introduced to cover the shell, the pressure restored, and the pieces quickly wiped and weighed. On account of the small quantity of water retained and its rapid evaporation, reliable results could not be obtained. A simple test was then devised to indicate whether or not the shell was porous. The principle of the test is to place a drop of dye solution on one side of the shell and to reduce the pressure on the other side to about half an atmosphere. With porous shells the dye is rapidly sucked through the shell over the whole of the exposed surface, whilst no colour passes through when the shell is non-porous. The apparatus used is shown in the diagram, but several other forms could equally well be used. It consists of a piece of glass tubing, about 5 cm. long and 5 mm. in internal diameter, which can be inserted in a rubber bung fitted to the end of a similar piece of glass tubing about 20 cm. long. The other end of the longer tube is connected with a small mercury reservoir by means of rubber tubing. In applying the test a small piece of shell is freed from the membranes on the inside by soaking in water for a few seconds, gently scraping with a knife, and rubbing between the thumb and fingers. It is then sealed to one end of the short piece of glass tubing by means of a tight joint of paraffin wax. The other end of the tube is inserted in the rubber bung after the reservoir has been so adjusted that the mercury stands approximately at the mark A, which is about 10 cm. below the egg shell. One or two drops of a 0.1 per cent. solution of methylene blue in 30 per cent. alcohol are placed on the top of the shell, and the mercury reservoir lowered until the mercury level stands at the mark B, about 20 cm. below the egg-shell. It is immaterial from the point of view of the success of the test whether the tube is attached to the outer or inner side of the shell, but, for convenience in holding the dye solution, it is better to attach it to the outer side. This can readily be done by placing the shell on a level surface, carefully resting the glass tube upon it, and painting molten paraffin wax on the joint with a small brush.



By means of this test over fifty new-laid eggs from different parts of the country, and over sixty eggs known to have been preserved, have been examined. Every new-laid egg showed colour penetrating the shell in 5 seconds or less, and in 30 seconds the whole surface was flooded. The penetration in the exposed circle, of about 5 mm. diameter, takes place at numerous spots—twenty or more—and the droplets of dye solution quickly unite. In the case of preserved eggs either no penetration took place after several minutes or else very slight colour was visible at an isolated spot or two. As a working test one minute was taken as the limit of time when the small tube was removed for examination. By gently warming the glass tube it can be removed from the shell, leaving the

wax-surrounded circle of shell intact. With several of the preserved eggs the test remained overnight under reduced pressure, and no trace of colour passed through the shell.

**ALKALINITY OF THE SHELL.**—Since both silicate and lime solutions are strongly alkaline, it was thought possible that eggs preserved in them might be differentiated from other eggs by tests for alkalinity, since, even if the shells were washed, traces of the preserving medium might remain. Ground egg-shells were tested with a universal indicator, and the shells of both preserved and unpreserved eggs reacted in practically the same way as well-washed precipitated calcium carbonate. Unopened eggs were soaked in warm water, and the *pH* of the poured-off liquid tested. This varied from 8 to 10 for both preserved and unpreserved eggs, and did not appear to be significant.

**EXAMINATION FOR TYPE OF PRESERVATION.**—(1) *Oiled Eggs.*—All the eggs of this type which could be obtained had been treated with oil, or with oil and wax mixtures. In every case sufficient of the preserving material remained to affect the "wetting" power of the shell. If a drop of water is placed on an unpreserved shell it wets the surface, and, when rubbed with a glass rod, the wetted film spreads. Silicated and limed eggs behave similarly. With oiled eggs the surface is not wetted, and the drop can be completely rolled over the shell surface. A drop of 95 per cent. alcohol remains as a globule on oiled eggs, whilst on other eggs it quickly spreads over the surface. Even if the eggs are well wiped with a dry cloth to remove as much surface oil as possible the effect remains. Hence it appears that if there is sufficient oil to seal the pores, its presence materially alters the interfacial tension and can be demonstrated.

By soaking oiled eggs in a suitable solvent a weighable quantity of the residual preserving medium can usually be obtained. Unopened unoled eggs, washed with decalin, gave 2 to 3 mgrms. of soluble matter, whilst oiled eggs similarly treated yielded from 9 to 38 mgrms. Attention must here be called to the possibility of eggs becoming accidentally contaminated with grease. It may be necessary to examine several portions of the shell for porosity to exclude this possibility. Adventitious grease does not usually seal pores except in small patches, and by drawing a coloured solution through the shell from the inside to the outside it will ooze round particles of grease not actually in the pores.

Attempts have been made to employ an oil-soluble dye for distinguishing this type of preservation. A drop of a solution of the dye in strong alcohol was placed on the shell and allowed to evaporate. The shell was then placed in 50 per cent. alcohol to see how easily the stain was washed off. In some cases the oiled eggs had fixed the colour, but, owing to the conglobation of the alcoholic solution on their surfaces, the colour was always more concentrated than on unoled eggs. In fact, the behaviour of the drop of alcoholic dye solution appeared more certain than the retention of colour after washing the stain with 50 per cent. alcohol. If sufficient of the oil remains to absorb dye, it shows by its surface action.

(2) *Silicated Eggs*.—For the characterisation of this type of preservation two lines of attack suggested themselves, namely, (a) the possible action of the alkaline silicate solution on the albuminous material in the shell basis, whereby the adsorption of dyes by the shell might be facilitated, and (b) the detection of silica.

With regard to (a) several dyeing tests were carried out, and it appeared that certain dyes were capable of being adsorbed to a much greater extent on silicated than on other shells. The intensive coloration appeared as a surface layer on the outside of the shell, whereas if the albuminous layers had been attacked it would have been expected that the dye would have penetrated below the surface. The most suitable dye for the purpose of this test was methylene blue, the same solution as that used for the porosity tests. A small portion of the shell, after removal of the adhering membranes, was boiled with the dye solution for about 5 seconds and then washed. Silicated shells give a deep indigo-blue colour on the outside of the shell, the inside being light blue. Other shells give the same light blue colour on the inside, but the outside is pale bluish with white eggs and greenish with brown eggs. The colour produced on the shell is due to a surface deposit of silica, which strongly adsorbs the dye. The same indigo-blue colour is produced by boiling precipitated silica with the dye solution, filtering and washing, and this may be used as a colour standard for comparing tests on egg-shells. Other useful dyes for giving distinguishable colour tests are malachite green and methyl violet.

(b) For the detection of silica, the shell was soaked in water, and the poured-off solution tested for soluble silica with molybdic acid. It was found most convenient to cover an unopened egg with water at about 40° C., and, after 15 minutes, to pour off the water and add 2 ml. of molybdic acid solution (2 grms. of ammonium molybdate and 40 ml. of *N* sulphuric acid to 100 ml.). The yellow colour produced after standing 15 minutes was matched by diluting 0.07 per cent. picric acid solution, 1 ml. of which in 50 ml. of water gives a colour approximately equal to that given by 1 mgrm. of silica. When either unpreserved, oiled, or limed eggs were so treated, the silica found never exceeded 0.2 mgrm. per 100 ml. of washing solution; with silicated eggs approximately 10 times this quantity was obtained. By repeating the soaking, silicated eggs gave further quantities of soluble silica, whilst other eggs gave negligible traces. Very dirty new-laid eggs gave no more silica than clean ones. The scrapings from two different chicken-runs were soaked in warm water and filtered. The filtrate tested with molybdic acid gave practically no colour.

These tests are sufficient to establish whether a preserved egg has been silicated or not.

(3) *Limed Eggs*.—The Board of Agriculture Leaflet No. 83, May, 1910, on the Preservation of Eggs, states that limed eggs can easily be recognised by the roughness of the shell. The 32 samples examined were very little different in texture from unpreserved eggs, and the reduction of the slight shininess or bloom usually present in unpreserved eggs was insufficient to render this distinction reliable.

Since the shell becomes non-porous by remaining in lime-water, it appears that the sealing agent must be calcium carbonate deposited there, and some means are required for differentiating between deposited carbonate and the material of the shell. For this purpose chemical tests seem useless. It was thought that a difference in the rate of solubility might be detectable, and the soluble lime was determined after soaking unopened eggs in water. As much was obtained from unpreserved and silicated eggs as from limed eggs.

The temperature of a hen is in the neighbourhood of 40° C., and the shell basis has been formed at this temperature. Any calcium carbonate deposited in the pores will have been produced at atmospheric temperatures which will almost certainly be below 20° C., and will generally be much lower, as care is taken in storing eggs in preserving solutions to keep the temperature as low as convenient. Consideration was therefore given to the possibility of distinguishing calcium carbonates formed at different temperatures. It is generally stated that when calcium carbonate is precipitated below 30° C., calcite is formed; above 70° C., aragonite is produced; between these temperatures a mixture of the two isomorphs is obtained. Johnston, Merwin and Williamson (*Amer. J. Sci.*, 1916, [IV], 41, 473) state that at 60° C. another form, referred to as  $\mu\text{CaCO}_3$ , results. This has a specific gravity of 2.54, the respective values for calcite and aragonite being 2.71 and 2.88. The specific gravity of air-dried ground egg-shell has been found to be 2.50, a value which is, of course, dependent on the proportions of organic and mineral matter. Microscopic examination of egg-shells revealed no characteristic crystalline forms, and a careful scrutiny of the surface and of scrapings of several limed eggs showed no recognisable calcite crystals.

Various qualitative tests have been described for the differentiation of calcite and aragonite, e.g. the cobalt nitrate test of Meigen (*Centr. Min.*, 1901, 577), the ferrous sulphate test of Panebianco (*Z. Kryst.*, 1904, 40, 288), and of Diesel (*Z. Kryst. Min.*, 1911, 49, 250), the dyeing tests of Thugutt (*Chem. Zentrbl.*, 1910, ii, 1084). It is generally considered that such tests are to be interpreted very carefully before being regarded as specific for either of the isomorphous forms (see Johnston, Merwin and Williamson, *loc. cit.*). The tests might, however, have some value for the problem under consideration. Calcium carbonate was therefore prepared by passing carbon dioxide into lime-water at 40° C., and a second preparation was made in the same way at 15° C. Portions were tested by boiling with reagents for a short time, filtering through a Gooch crucible, and washing. Of the substances specified in the above papers, only Congo red gave a marked difference with the two carbonates. The one precipitated at 40° C. gave a bright orange-red colour, whilst that formed at 15° C. gave but a faint flesh-pink. Suitable conditions for applying the test were to employ a 0.1 per cent. aqueous solution of Congo red and to boil for about 5 seconds. The test was then applied to portions of egg-shells, and it was found that unpreserved eggs gave a good orange-red colour on both the outside and inside of the shell, of practically the same intensity as that obtained from the carbonate precipitated at 40° C. The colours produced on the

outside of white and brown eggs were almost identical, the depth of colour covering the normal differences in tint. Limed eggs gave the same colour as unpreserved eggs on the inside of the shell, but the outsides were considerably lighter. The colour varied from a very pale flesh colour to an orange-red and was sometimes irregularly distributed. The test has been applied to all the eggs available, including over 50 new-laid eggs and 32 limed eggs. The colours produced were assessed by comparison with a scale obtained by applying the test to calcium carbonate precipitated at 40° C., and diluting the washed and dried coloured product with undyed calcium carbonate; they were as follows:

Colour shade.				New-laid eggs.	Limed eggs.
Full ..	..	..	..	All	None
Three-quarters	..	..	..	—	12
Half	..	..	..	—	14
Quarter	..	..	..	—	6

The carbonate precipitated at 15° C. gave a colour about equal to the quarter standard.

Other dye solutions were tried, and similar results were obtained with alizarine S (0.1 per cent. aqueous solution) and the sodium salt of alizarine (0.1 grm. of alizarine and 5 ml. of *N*/10 sodium hydroxide solution to 100 ml.). With alizarine S both the outside and inside of unpreserved egg-shell were coloured purplish red to approximately the same extent; limed eggs gave a similar colour on the inside, but the outside was considerably lighter. In the case of the sodium salt of alizarine the unpreserved shell adsorbed much more colour on the outside than on the inside, the outside having a blackish lustre. This lustre effect was absent from the shells of limed eggs similarly treated, the outside being again deeper than the inside, but considerably lighter than the outside of unpreserved shell.

These colour tests have been applied to oiled and silicated eggs. All the oiled eggs reacted similarly to unpreserved eggs, except that with the sodium salt of alizarine the blackish lustre was only slightly visible. Silicated eggs, however, reacted like limed eggs, the film of deposited silica evidently protecting the basis of the shell.

The above-described dyeing tests do not, therefore, distinguish between limed and silicated eggs. But, as already indicated, the silicated eggs can be identified by other means. A non-porous egg which fails to absorb dyes when tested as described above, and gives no reactions for silica cannot be affirmed, definitely, to be limed, yet the statement can be made that the composition of the shell has been altered, and that it reacts in the same way as limed eggs and probably has been limed.

**GENERAL METHOD OF EXAMINATION.**—From the tests which have been developed the following procedure has been adopted for the examination of suspected eggs. With samples of less than one dozen eggs, at least half the



number are placed in separate 150 ml. beakers; with larger samples a representative number is taken. The eggs are covered with warm water at about 40° C., note being made of the surface behaviour as the water is poured on each egg. The water is maintained at about 40° C. for 10 to 15 minutes, and is then poured off, and to each solution is added 2 ml. of molybdic acid solution (2 grms. of ammonium molybdate and 40 ml. of *N* sulphuric acid to 100 ml.). After standing about 15 minutes the colour, if any, is matched with that given by adding picric acid solution (0.07 per cent.) to a similar quantity of water. If not more than about 0.2 ml. of picric acid solution is required, the dissolved silica is regarded as normal. Each egg is then broken or blown and the contents discarded. Portions of the shell, about  $\frac{1}{2}$  inch by  $\frac{1}{2}$  inch, are placed in water, and the egg and shell membranes removed by gentle scraping and rubbing. It may be mentioned that the egg membrane can generally be peeled off easily, but the shell membrane adheres closely to the shell. Two or three pieces of the shell from each egg are then tested for porosity. Complete absence of porosity or very slight porosity, in all the pieces tested is regarded as evidence of preservation. Further portions of each shell are then tested by boiling for about 5 seconds with (1) methylene blue solution (0.1 per cent.) and (2) Congo red solution (0.1 per cent.) and then washing. If care is taken to prevent overlapping, several pieces of shell may be boiled at the same time with either reagent, including pieces of unpreserved egg-shell used as controls. Other portions of shell are tested for surface action with a drop of strong alcohol.

According to the results obtained, further tests, as described above, may be tried, and other eggs from the sample may be tested.

SUMMARY.—(1) Unpreserved eggs are porous, whilst eggs preserved in oil, water-glass, or lime-water, are non-porous.

(2) Oiled eggs retain traces of oil in the pores, which give marked surface effects, but which do not prevent normal adsorption of dyes by the shell.

(3) Silicated eggs have a surface-film of silica which seals the pores, and which can be detected by extraction and by its behaviour towards certain dyes.

(4) Limed eggs have a surface-film of deposited calcium carbonate, which seals the pores and which reacts differently towards certain dyes from the normal calcium carbonate of the shell.

(5) A test for porosity of the shell, and a method for the examination of eggs suspected of being preserved are described.

I wish to thank the Government Chemist for permission to publish this paper.

## The Determination of Soluble Solids in Jams, etc.

A COMMUNICATION FROM THE BRITISH ASSOCIATION OF RESEARCH FOR THE COCOA, CHOCOLATE, SUGAR CONFECTIONERY AND JAM TRADES.—T. MACARA, F.I.C., *Director of Research*.

IN the Standards for Jams (ANALYST, 1930, 55, 694) a minimum limit for soluble solids was included. It is exceedingly difficult to determine the true percentage of soluble solids, and the limit was, therefore, coupled up with a covering definition. This reads as follows:—

"All jams, whether of First Quality or Second Quality shall contain not less than 68½ per cent. total soluble solids, by refractometer reading when cold, uncorrected for insoluble solids."\*

It will be obvious at a glance that the solids determined in this manner may be 2 or 3 per cent. higher than the truth if the insoluble solids are high. There are other errors which, in some cases, cancel each other to a greater or less extent, for the reading is taken either direct in percentages of sucrose, or is converted into these from the refractive indices by means of tables or the corresponding factors. As the jam contains invert sugar, acids and salts, all having refractive indices differing from that of sucrose, the soluble solids determined in this manner may be 1 or 2 per cent. wide of the truth; *e.g.* invert sugar, when present in large proportions, gives a low result.

This method of determining the soluble solids, which was adopted by the Public Analysts' Committee and the Food Manufacturers' Committee jointly, has many advantages from the practical standpoint, and is more satisfactory to all concerned than leaving it open to all parties to select their own methods.

The advantages of this system may be summarised briefly. In the first place the refractometer has been used for a comparatively long time to control the composition of the jam at the time of manufacture, the determination of the true soluble solids by any other means taking much too long. Further, refractometers are in general use, and the taking of this reading is simple, compared with other methods. In this way both manufacturers and analysts will be using the same methods.

In this connection there is, however, one objection to the method, *viz.* that only a very small portion of the jam is tested, and, as jam is by no means a homogeneous mixture, results which do not correspond with that of the carefully averaged sample may readily be obtained.

For example, the surface layer of an old jam may give a reading as much as 8 per cent. or more above that of the bulk of the jam. If, in testing such a sample, care is not exercised to see that the whole sample is carefully mixed, wrong results may be obtained. The same remarks apply in a less degree to layers of the jam all the way down the jar. This effect is due to the fact that under normal storage conditions the evaporation of moisture from the surface is more rapid than its

\* The "cold" temperature is taken as 20°C. in the laboratory of the Association.—T.M.

diffusion upwards from the lower portions. These remarks also apply to whole-fruit jams in which the pieces of fruit may have a different soluble solids content from that of the jelly portion.

This objection is easily overcome if the mixing of the sample is sufficiently thorough, all the whole fruit being well crushed, or, preferably, the entire sample being passed through a small mincer.

Refractometers are now made with scales giving both refractive indices and corresponding sugar percentages. Messrs. Bellingham and Stanley also make a butyro-refractometer which contains a scale showing both the usual numbers and the corresponding sugar percentages. As, however, these instruments are of a special type, the following table for use with the ordinary types will be found convenient in testing jams, syrups, etc.

The table has been prepared by C. L. Hinton, who has given the corresponding sugar values for both the refractive indices and butyro refractometer numbers. The butyro-refractometer numbers equivalent to the indices have been taken from the table given in E. R. Bolton's "Oils, Fats and Fatty Foods." The scale of sugar percentages from 61 to 75 is based on data arrived at experimentally by Hinton in the Association's laboratory. The higher percentages are based on Main's tables.

Hinton's scale between 61 and 66 agrees closely with that of Schönrock, but differs somewhat from Main's at the lower concentrations. Main's figures for the higher concentrations are believed to be reasonably reliable.

It should be noted that while temperature affects the reading, a difference of 5° C. only produces an error of 0.35 per cent. in the actual solids.

TABLE I.

$n_D^{20}$	Sugar. Per Cent.	Butyro ref. scale.	$n_D^{20}$	Sugar. Per Cent.	Butyro ref. scale.	$n_D^{20}$	Sugar. Per Cent.	Butyro ref. scale.
1.4440	61.1	28.3	1.4500	63.6	36.7	1.4560	66.2	45.2
2	.1	.6	2	.7	37.0	2	.3	.5
4	.2	.9	4	.8	.2	4	.4	.7
6	.3	29.2	6	.9	.5	6	.4	46.0
8	.4	.4	8	64.0	.8	8	.5	.3
50	.5	.7	10	.1	38.1	70	.6	.6
2	.6	30.0	2	.2	.3	2	.7	.9
4	.7	.3	4	.2	.6	4	.8	47.2
6	.7	.6	6	.3	.9	6	.9	.5
8	.8	.8	8	.4	39.2	8	.9	.7
60	.9	31.0	20	.5	.5	80	67.0	48.0
2	62.0	.4	2	.6	.7	2	.1	.3
4	.1	.6	4	.7	40.0	4	.2	.6
6	.2	.9	6	.8	.3	6	.3	.9
8	.2	32.2	8	.9	.6	8	.4	49.2
70	.3	.5	30	.9	.9	90	.4	.5
2	.4	.8	2	65.0	41.1	2	.5	.8
4	.5	33.0	4	.1	.4	4	.6	50.1
6	.6	.3	6	.2	.7	6	.7	.4
8	.7	.6	8	.3	42.0	8	.8	.7
80	.8	.9	40	.4	.3	1.4600	.8	51.0
2	.9	34.2	2	.4	.5	2	.9	.3
4	.9	.4	4	.5	.8	4	68.0	.6
6	63.0	.7	6	.6	43.1	6	.1	.9
8	.1	35.0	8	.7	.4	8	.2	52.2
90	.2	.3	50	.8	.7	10	.3	.5
2	.3	.6	2	.9	44.0	2	.3	.8
4	.4	.8	4	.9	.3	4	.4	53.1
6	.5	36.1	6	66.0	.6	6	.5	.4
8	.6	.4	8	.1	.9	8	.6	.7

TABLE I.—*continued.*

$n_D^{20}$	Sugar. Per Cent.	Butyro ref. scale.	$n_D^{20}$	Sugar Per Cent.	Butyro ref. scale.	$n_D^{20}$	Sugar. Per Cent.	Butyro ref. scale.
1.4620	68.7	54.0	1.4720	72.8	69.5	1.4820	76.8	86.4
2	.8	.3	2	.9	.9	2	.9	.7
4	.9	.6	4	73.0	70.2	4	77.0	87.1
6	.9	55.0	6	.1	.5	6	.1	.5
8	69.0	.3	8	.1	.8	8	.1	.8
30	.1	.6	30	.2	71.1	30	.2	88.2
2	.2	.9	2	.3	.4	2	.3	.5
4	.3	56.2	4	.4	.8	4	.4	.9
6	.4	.5	6	.5	72.1	6	.4	89.2
8	.4	.8	8	.6	.4	8	.5	.6
40	.5	57.1	40	.6	.7	40	.6	90.0
2	.6	.4	2	.7	73.0	2	.7	.3
4	.7	.7	4	.8	.3	4	.8	.7
6	.8	58.0	6	.9	.7	6	.8	91.1
8	.9	.3	8	74.0	74.0	8	.9	.4
50	.9	.6	50	.0	.3	50	78.0	.8
2	70.0	.9	2	.1	.6	2	.1	92.1
4	.1	59.2	4	.2	75.0	4	.2	.5
6	.2	.5	6	.3	.3	6	.2	.9
8	.3	.8	8	.3	.6	8	.3	93.2
60	.4	60.2	60	.4	76.0	60	.4	.6
2	.4	.5	2	.5	.3	2	.5	94.0
4	.5	.8	4	.6	.7	4	.6	.3
6	.6	61.1	6	.7	77.0	6	.6	.7
8	.7	.4	8	.7	.3	8	.7	95.0
70	.8	.7	70	.8	.7	70	.8	.4
2	.9	62.0	2	.9	78.1	2	.9	.8
4	.9	.4	4	75.0	.4	4	.9	96.1
6	71.0	.6	6	.1	.7	6	79.0	.5
8	.1	.9	8	.1	79.1	8	.1	.9
80	.2	63.2	80	.2	.4	80	.2	97.2
2	.3	.5	2	.3	.8	2	.3	.6
4	.3	.8	4	.4	80.1	4	.3	98.0
6	.4	64.2	6	.5	.5	6	.4	.3
8	.5	.5	8	.6	.8	8	.5	.7
90	.6	.8	90	.6	81.2	90	.6	99.1
2	.7	65.1	2	.7	.5	2	.7	.4
4	.8	.4	4	.8	.9	4	.7	.8
6	.8	.7	6	.9	82.2	6	.8	
8	.9	66.1	8	76.0	.5	8	.9	
1.4700	72.0	.4	1.4800	.0	.9	1.4900	80.0	
2	.1	.7	2	.1	83.2	2	.0	
4	.2	67.0	4	.2	.6	4	.1	
6	.2	.3	6	.3	.9	6	.2	
8	.3	.7	8	.3	84.3	8	.3	
10	.4	68.0	10	.4	.6	10	.3	
2	.5	.3	2	.5	85.0	2	.4	
4	.6	.6	4	.6	.3	4	.5	
6	.7	.9	6	.6	.7	6	.6	
8	.7	69.2	8	.7	86.0	8	.6	

## THE DETERMINATION OF "TRUE" SOLUBLE SOLIDS.

Although the percentage of true soluble solids is not required in the analysis of jams in connection with the maintenance of the Standards, this figure may sometimes be of interest to analysts. In such cases, the following notes on the subject may be helpful:

The determination of the solids by drying methods does not yield satisfactory results, as the acids present in the jam not only invert the sugar, but also tend to

decompose it. Further, the sugars, particularly laevulose, do not readily part with the last traces of moisture.

Distillation methods, employing volatile non-miscible liquids, would probably be equally unsuitable, for the same reasons.

The best methods would appear to be indirect ones, depending on either the refraction of the jam itself or on that of a solution, or on the gravity of this solution, using suitable factors for calculating the percentage of sugar present, and making the necessary corrections for the effects of the insoluble solids, acids, etc., in the jam. These methods have been worked out in great detail in the Association's laboratories, and the tables given below have been computed from data arrived at experimentally.

BY DIRECT REFRACTOMETER READING.—As already pointed out, there are difficulties in obtaining from a minute portion of the sample a reading which will give a result of the desired accuracy and truly representative of the bulk of the jam. With the necessary care, this can, however, be attained. The reading so obtained is then converted into percentage of sugar by means of Table I, and afterwards corrected for the errors due to the different refractive values of invert sugar, glucose (if present) and acid; this result is then corrected for the effect of the insoluble solids.

In order to simplify these calculations a table is given below from which the corrections for invert sugar, glucose and acid can be readily computed, and an example is given to illustrate the method.

TABLE II.

Corrections for Invert Sugar, Glucose Solids and Acids to be used in Calculating Total Soluble Solids from the direct reading on the Refractometer.

Substance.	Percentage in sample.	Correction.
Invert sugar .. .. .	20	+0.45
	40	+0.90
	60	+1.35
Glucose solids .. .. .	20	-0.25
	40	-0.5
Citric acid .. .. .	1	+0.1
	2	+0.2
	3	+0.3

*Example I.*—A sample of black currant jam gave the following results on analysis:

Insoluble solids .. .. .	2.0 per cent.
Invert sugar .. .. .	31.0 " "
Acidity as citric acid .. .. .	0.95 " "
Direct refractometer reading	$n_D^{20} = 1.4673$

*Calculation.*— $n_D^{20}$ , 1.4673, corresponding with 70.9 soluble solids as sucrose.

Correction for invert sugar,	+ 0.7
" " acid,	+ 0.1

Total 71.7

This is corrected for insoluble substances thus:

$$\frac{71.7 \times (100 - 2.0)}{100} = 70.3 \text{ per cent.}$$

**DETERMINATION OF SOLUBLE SOLIDS BY SPECIFIC GRAVITY METHOD.**—In this case a 20 per cent. solution of the jam is made by dissolving 50 (or 100) grms. of the jam.\* The solution and fibre are transferred to a 250 (or 500) ml. flask and made to volume at 20° C. The solution is next filtered through muslin or a fine sieve and the gravity determined as usual at 20° C.

The percentage of solids in solution is then calculated as sucrose, using the factor 3.845, which is that which has been found to be correct for sugar solutions of this concentration and at 20° C. The solids are then calculated to percentage in original sample and corrected for invert sugar, etc., using the corrections given in Table III, and afterwards for volume of insoluble, as shown in the following example:

TABLE III.

Corrections to be used where solids are determined in 20 per cent. solution at 20° C. and the solution factors 3.845 and 1.428 are used for calculating the solids obtained by gravity and dipping refractometer respectively.

Substance.	Apparent percentage in sample.	Correction on solids	
		by sp. gr. at 20°/20° C.	by dipping re- fractometer 1.20.
Invert sugar .. ..	20	-0.07	+0.22
	40	-0.14	+0.44
	60	-0.21	+0.66
Glucose solids .. ..	20	-0.34	-0.46
	40	-0.68	-0.92
Citric acid .. ..	1	-0.09	+0.12
	2	-0.18	+0.24
	3	-0.27	+0.36

*Example II.*—Black currant jam as in I.

Sp. gr. of 20 per cent. solution = 1.05423

Soluble solids as sucrose  $\frac{54.23}{3.845} \times \frac{100}{20} = 70.5$  per cent.

Correction for invert sugar, = — 0.1    "    "  
 "    "    acid,    = — 0.1    "    "

Total sugar    70.3

This has to be corrected for the volume of the insoluble matter in the solution, and for this purpose it has been found to be satisfactory to assume that its gravity is 1. Actually the gravities of the various insoluble matters of fruit vary from about 1.4 to 1.8, but there appears to be a compensating error which is thought to be due to adsorption of the sugars. In any case the error, if any, is small. In the present instance the percentage corrected in this way is:

$$\frac{70.3 \times (100 - 0.4)}{100} = 70.0 \text{ (cf. result in Example I.)}$$

**DETERMINATION BY MEANS OF THE DIPPING REFRACTOMETER.**—The 20 per cent. solution is prepared as for the gravity and the reading is taken at 20° C. This instrument gives more exact readings than does the Abbé instrument. The reading is, however, on an arbitrary scale, which has then to be converted to

\* Care should be taken when dissolving the jam to keep the solution at a sufficiently low temperature to prevent inversion of the sugar.

indices. A table for this purpose is given in Leach's "Food Inspection and Analysis" (Fourth edition, p. 102). The refractive index so obtained is then converted to sugar percentage by deducting the refractive index of water, multiplying by 1000, dividing by the solution factor 1.428, which has been found to be the appropriate one for sugar solutions of this concentration and temperature, and, finally, bringing to percentage in original sample.

The necessary corrections are made as before (see fourth column, Table III).

*Example III.*—Black currant jam (analysis as in Example I).

$n_D^{20}$  of 20 per cent. solution = 1.35302.

Soluble solids as sucrose:

$$\frac{(1.35302 - 1.3330) \times 1000}{1.428} \times \frac{100}{20} = 70.1$$

Correction for invert sugar	+	0.3
,,       ,,   acid	+	0.1

Total           70.5

Correction for insoluble substances as in Gravity Method:

$$\frac{70.5 (100 - 0.4)}{100} = 70.2.$$

If the three results are compared it will be noted that there is a maximum difference of 0.3. This is probably due mainly to the different effects of the salts and possibly of other substances in solution on the refractive index and gravity respectively. It is, therefore, usual to take the mean of the results obtained by the two methods, particularly where the refractive index has been taken by the dipping refractometer.

Some jams show greater differences between the results obtained by the two methods than others. Black currant is one of the worst in this respect, probably owing to the fact that it contains more salts and that it is more highly coloured than most of the others. Results for jam such as strawberry and apple frequently agree very closely, *i.e.* within 0.1 per cent.

Where it is desired to obtain approximately the true percentage of soluble solids, when the percentages of invert sugar and acid are unknown, the gravity method will give the best results, *i.e.* without applying the necessary corrections for these ingredients. A glance at the tables of corrections will show that these are smallest when using this method, and, in the case of the example, would have made an error of 0.2 at most, provided the correction for insoluble solids was made.

**Errata.**—In the May issue, p. 296, l. 15, for "0.2 mgrm." read "0.2 grm."

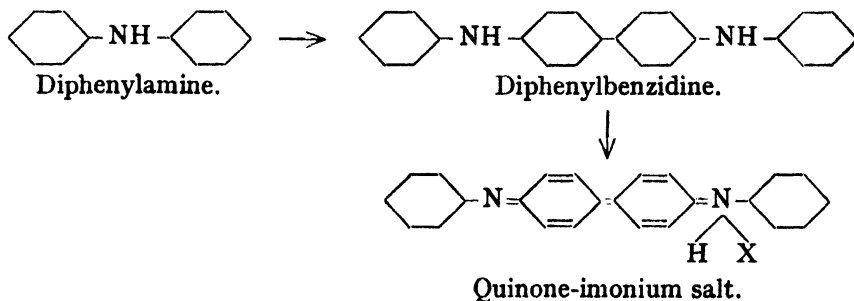
**The Detection of Benzoic Acid**, p. 303: The legends beneath the lower block should be reversed. "C" represents silver salicylate; "D" silver benzoate.

## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### THE NITRATE TEST FOR THE DETECTION OF ADDED WATER IN MILK.

In several communications which have appeared from time to time in *THE ANALYST*, the use of diphenylamine is recommended as a test for nitrates in milk. (G. D. Elsdon and J. A. L. Sutcliffe, *ANALYST*, 1913, **38**, 450; G. D. Elsdon and P. Smith, *ANALYST*, 1922, **47**, 18; A. F. Lerrigo, *ANALYST*, 1931, **433**; D. R. Wood, E. T. Illing and A. E. Fletcher, *ANALYST*, 1931, 248.) This test is more sensitive if diphenylbenzidine be substituted for diphenylamine. F. Kehrmann and St. Micewicz (*Ber.*, 1912, **45**, 2641) showed that the blue compound formed by the action of nitric acid on diphenylamine is a quinone-imonium salt of diphenylbenzidine. The action takes place in two stages, firstly, the oxidation of diphenylamine to diphenylbenzidine; and secondly, the oxidation of diphenylbenzidine to a quinone-imonium salt:



The first stage of the oxidation is effected at the expense of some of the nitrate present. If the milk contains only traces of nitrate, or if too much diphenylamine be used, it may happen that no nitrate is left to effect the second stage of oxidation and to produce the blue colour.

The preliminary stage of oxidation can be avoided by using ready-made diphenylbenzidine, in which case the whole of the nitrate present is available for the second stage of oxidation, and the test becomes much more delicate and certain (*cf.* E. A. Letts and F. W. Rea, *J. Chem. Soc.*, 1914, **105**, 1157; L. Smith, *Z. anal. Chem.*, 1917, **56**, 28).

In my experience diphenylamine cannot be relied upon to detect nitrate in concentrations less than one part per million, but with diphenylbenzidine a good gradation of colour is obtained with amounts of nitrate ranging from 0.1 to 1 part per million.

Diphenylbenzidine is readily prepared from diphenylamine by Marquayrol and H. Muraour's method (*Bull. Soc. Chim.*, 1914, [iv], **15**, 186). This method is not described in detail in the abstracts available. Eight grms. of diphenylamine, dissolved in 20 c.c. of glacial acetic acid, are poured into a mixture of 50 c.c. of



concentrated sulphuric acid and 150 c.c. of water which has previously been cooled to room temperature. To this solution is added, in small portions at a time, with shaking, a solution of 4.7 grms. of sodium dichromate in a mixture of 200 c.c. of water and 29 c.c. of dilute sulphuric acid (1 vol.  $\text{H}_2\text{SO}_4$  + 4 vols. water). The solution becomes deep blue and a precipitate is formed, the temperature rising slightly. The liquid is shaken for a few minutes and then poured into a 2-litre flask containing 100 c.c. of a strong solution of sodium bisulphite (sp. gr. about 1.23) and 400 c.c. of water. A voluminous dark green precipitate is formed. The mixture is heated on a steam bath until the precipitate turns brown (about 60° to 70° C.). It is then allowed to cool and is filtered. The precipitate is washed with water and then with alcohol to remove unchanged diphenylamine, dried and crystallised from hot toluene. The yield of diphenyl-benzidine is about 60 per cent. of the theoretical amount. The product is more or less grey in colour, but may be used without further purification for the nitrate test. The technique of this test is the same as when diphenylamine is used.

G. W. MONIER-WILLIAMS.

## Official Appointments.

THE Minister of Agriculture and Fisheries has confirmed the following appointments:

ROWLAND H. ELLIS, F.I.C., as Agricultural Analyst for the County of Herefordshire (May 9, 1931).

ERIC VOELCKER, A.R.C.S., F.I.C., as Deputy Agricultural Analyst for the County of Northumberland (May 9, 1931).

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### "CREAM" IN CONFECTIONERY.

J. LYONS & CO. v. KEATING.

ON February 6th the High Court remitted this case to the Swindon justices for them to consider whether the articles in question were sold as substances purporting to be cream as defined in the Artificial Cream Act, 1926 (ANALYST, 1931, 253). After a further hearing on March 9th, 1931, the justices found that the articles were sold as substances purporting to be cream as defined by the Act, and that there was evidence to support such a finding. An appeal against this finding was heard in the King's Bench Division (before the Lord Chief Justice, Mr. Justice Avory and Mr. Justice Charles) on May 7th, 1931. Sir John Simon, K.C., Mr. Roland Oliver, K.C., and Mr. C. Salmon appeared for the appellants, and Mr. G. D. Roberts and Mr. Elam for the respondents.

The Lord Chief Justice (Lord Hewart) said that he had come to the conclusion that the appeal ought to be allowed. When he looked at the title and the contents of the statute he thought that it was tolerably plain that the statute was dealing with the sale of cream or artificial cream *simpliciter*. The words were a little curious, but what was being considered and dealt with was a substance purporting to "be" cream or artificial cream; not "a substance purporting to contain" cream. If the Act had meant the latter, it would have been easy to say "a substance or article purporting to be or to contain cream." Whether that construction was what the Legislature meant to express he did not know, but it was what the Legislature had expressed. It might be very unfortunate, and, in some cases, entirely misleading, that composite articles should be sold under names including the word cream. But such gaps could only be filled by the Legislature and not by that Court. He thought that the argument of the appellants that the Act applied only to cream as a separate commodity was right. The question whether it was desirable that the word cream should be applied to confectionery of that sort might well receive attention; for nothing could be more misleading than the titles given to the articles in this case.

Mr. Justice Avory and Mr. Justice Charles gave judgment to the same effect, and the appeal was allowed.

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### ALLEGED DERMATITIS FROM METAL.

#### SHARP v. SALMON & GLUCKSTEIN.

ON May 12 the plaintiff, a consulting surgeon, brought an action in the King's Bench Divisional Court (Mr. Justice Horridge) for damages for personal injuries alleged to have been caused by a wrist watch sold to him by the defendants.

Mr. Kingbury, for the plaintiff, said that after the watch had been worn for a few days on the wrist of the plaintiff there was intense irritation, and a rash spread up his arm, over the left side of his neck and across his brow.

The back of the watch, which was stamped "pure nickel," had been analysed, and found to be composed of 55 per cent. of copper, 13 per cent. of nickel, 31 per cent. of zinc, with traces of lead and tin, but no antimony. Far from being pure nickel, it was a variety of German silver, and was distinctly radioactive.

Mr. Hilbery, for the defence, suggested that the plaintiff had worn this watch tightly on his wrist and that this had set up the irritation.

Dr. Knowsley Sibley said that the plaintiff on June 18, 1929, was suffering from diffused dermatitis. In view of the analysis of the watch case, he was of opinion that the metal itself could not cause the dermatitis, which he attributed to pressure and sweating under the watch. He did not think that there would be enough radio-activity at the back of the watch to cause the trouble.

At the close of the evidence for the plaintiff the jury stopped the case, expressing the view that the watch was reasonably fit for ordinary use, and that the skin irritation was not caused by any defect of the watch, but by the wearing of it.

Judgment was entered for the defendants, with costs.

### CHEESE SANDWICHES.

ON April 23rd a firm was summoned at the North London Police Court for selling cheese sandwiches, the fat in the cheese of which contained 67 per cent. of fat other than milk fat.

Mr. Robertson, for the Islington Borough Council, said that the article between two biscuits making the sandwich was "margarine cheese," whereas cheese was defined by the Act as "the substance usually known as cheese, containing no fat derived otherwise than from milk."

The Magistrate dismissed the summons under the Probation of Offenders Act, but said that it would be a very much more serious matter if, after these proceedings, there should be any further breach of the law.

## Government of Bihar and Orissa.

### ANNUAL REPORT OF THE CHEMICAL ANALYST FOR THE YEAR 1930.

DURING the year there were examined 1527 samples of foods, drugs and waters received from 20 districts of 21 districts of the province.

**GHEE.**—Of 353 samples of ghee examined, 83 were samples of pure cow and buffalo ghee prepared in the laboratory by the native processes, with the object of testing the range of the Reichert-Wollny value and of the butyro-refractometer reading, the milk samples (from the individual cow or buffalo) being obtained through reliable officers from various parts of the province. The butyro-refractometer reading ranged from  $40^{\circ}$  to  $46^{\circ}$ , and the Reichert-Wollny value of buffalo ghee ranged from 41.2 to 26, and that of cow ghee from 35.4 to 15.5.

**RAPE OIL AND MUSTARD OIL.**—Of 237 samples examined, 95 were found to be adulterated or below the standard. The usual adulterants are linseed oil and niger-seed oil.

**SWEETS.**—Forty-six samples of sweets were examined, with a view to discover whether they were made with pure ghee or pure rape oil. Of 46 samples, 28 were found to have been from ghee or oil which was adulterated or below standard.

**TEA.**—Thirty-one samples of tea were examined, of which 27 were found to be adulterated or below standard.

**MISCELLANEOUS.**—Four samples of rice and 2 samples of pulse were examined in order to test their fitness for human consumption. These grains were preserved in tin and mercury amalgam. Two samples were found to be contaminated with mercury and tin amalgam, with lead as impurity.

J. C. DAS.

## General Medical Council.

### PHARMACOPOEIA COMMISSION: REPORTS OF SUB-COMMITTEES.

THE Pharmacopoeia Commission have published the Report of the Pharmaceutical Chemistry Sub-Committee and the Report of the Cod-liver Oil Colour Test Sub-Committee,\* and invite helpful criticisms and comments on the suggested standards and tests.

\* The booklet containing these reports may be obtained from the Office of the Pharmacopoeia Commission, 44, Hallam St., Portland Place, W.1. Price 3/-. The Cod-liver Oil Colour Test Report will be published *in extenso* in the July issue of THE ANALYST.—EDITOR.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

## Food and Drugs Analysis.

**Determination of the Calcium, Magnesium and Acid-Soluble Phosphorus of Milk by means of Trichloroacetic Acid Filtrates.** G. P. Sanders. (*J. Biol. Chem.*, 1931, 90, 747-756.)—A procedure is described for the determination of calcium, magnesium and acid-soluble phosphorus in cow's milk without ashing; it involves the trichloroacetic acid filtrate method. The elimination of the ashing method effects a saving of at least one working day in the preparation of the samples. Data are presented to show the accuracy of the method, as compared with the ashing method. It was first decided to eliminate the addition of water, in order to try to shorten the procedure and increase the degree of accuracy. A study was made of the use of equal parts of milk and acid solution. The calcium content of normal cow's milk was determined by the ashing method and the trichloroacetic acid method. One part of 10 per cent. acid solution was added to 1 part of milk. When skim milk was used, the filtration method produced results which were usually slightly high for calcium, not greater than 1.5 per cent.; when whole milk was used, the positive error was as great as 4.03 per cent. The results indicated an increased content of calcium in skim milk as compared with the same milk before skimming, and this increase corresponded closely with the percentage of fat in the whole milk. Finally, the use of 4 parts of 10 per cent. trichloroacetic acid solution with 1 part of milk was found to be most effective in the preparation of the protein-free filtrates for mineral determinations. A high degree of accuracy was attained in determinations of calcium and magnesium. In a number of milk samples, 68.4 to 81.9 per cent. of the phosphorus was found to be acid-soluble. There was no definite correlation between the amount of acid-insoluble phosphorus and the amount of casein; in general, however, milk high in casein was high in acid-insoluble phosphorus. The procedure should be of value in the study of phosphorus combinations in milk, with particular reference to casein and lipid phosphorus. The technique is as follows:—*Trichloroacetic Acid Filtrate Method.*—Twenty c.c. samples of cow's milk are pipetted into each of two 100 c.c. volumetric flasks, the pipette and flasks having been accurately calibrated. The flasks are filled to the 100 c.c. mark with 10 per cent. trichloroacetic acid solution, the acid being added slowly and the flask rotated constantly. The contents are thoroughly mixed and left stoppered for 30 minutes with frequent shaking. They are then filtered through dry 15 cm. acid-washed filter paper; the funnel is covered with a watch-glass during the filtration. From each sample 50 c.c. of the filtrate (corresponding with 10 c.c. of milk) are taken for the mineral determination. *Calcium.*—Calcium analyses are carried out by the method of Meigs, Blatherwick and Cary (*J. Biol. Chem.*, 1919, 37, 1), which is a modification of the McCrudden (*J. Biol.*

*Chem.*, 1911-12, 10, 187) method. Calcium is determined as calcium oxalate, by permanganate titration. The precipitation of the calcium oxalate may be accelerated by heating, for there is no appreciable amount of heat-coagulable protein present to cause interference in the filtration and washing of the crystals when the 4:1 ratio of acid and milk is used. *Magnesium*.—Owing to the small amount of magnesium which occurs in milk, 40 c.c. samples of milk are made up to 200 c.c. volumes with 10 per cent. trichloroacetic acid solution, and, after filtering, 150 c.c. aliquot portions, corresponding to 30 c.c. of milk, are used. Magnesium determinations are carried out by Method II of the Association of Official Agricultural Chemists ((11), p. 29); in the analyses of the trichloroacetic acid filtrates the first addition of alcohol is omitted. *Acid-soluble Phosphorus*.—The samples are prepared as described for calcium, with the use of trichloroacetic acid as the protein precipitant and mineral solvent. Phosphorus analyses in the filtrate are carried out by the gravimetric procedure of the Association of Official Agricultural Chemists ((11), p. 3). Trichloroacetic acid solution added to milk produces a much coarser precipitate than does the mixture of sodium tungstate and sulphuric acid of Folin and Wu; the trichloroacetic acid mixture filters much more rapidly and yields a considerably larger amount of filtrate in proportion to the amount of milk used.

P. H. P.

**Cause of Beet Odour and Taste in Milk and Butter.** P. Post. (*Z. Unters. Lebensm.*, 1931, 61, 171-174.)—The odour and taste of beet developed during October and November in milk and butter from districts where beet sugar is grown are not of bacterial origin, since they occur in milk obtained under conditions of controlled cleanliness to the same extent as in that obtained in the usual way. They are characteristic of cattle fed on beet-heads and leaves, and may be simulated by the addition of 10 mgrms. of trimethylamine hydrochloride to 100 grms. of milk. The trimethylamine (which probably originates from betaine) was separated from the tainted milk by distillation in the presence of lime-water, into hydrochloric acid; the distillate was evaporated, the residue extracted with absolute alcohol, the extract evaporated, and the residue examined. Tests for trimethylamine hydrochloride which are not given by ammonium chloride are: (a) the odour of trimethylamine on addition of sodium hydroxide, (b) a yellow precipitate with a solution of iodine in potassium iodide solution; (c) a white precipitate obtained from the solid salt with mercury potassium iodide solution. All gave positive results with 200 c.c. of the tainted and treated milks, and negative results with normal milk. It is considered impracticable to forbid the use of beet-fodder, though propaganda might restrict its use. As remedies, warming the milk under reduced pressure, and extraction of the butter first with water acidified with lactic or tartaric acid, and then with fresh water, have yielded promising results in the laboratory.

J. G.

**Some Properties of Honey Colloids and the Removal of Colloids from Honey by Bentonite.** R. E. Lothrop and H. S. Paine. (*Ind. Eng. Chem.*, 1931, 23, 328-332.)—The amount of colloid substances present in honey varies

between about 0.1 per cent. for light coloured honey and 1 per cent. for dark (buckwheat) honey, as determined by ultra-filtration through standardised colloidion membranes. These colloid constituents appear to influence such properties as colour, flavour, caramelisation point and crystallisation of the honey. When the pH value of honey is adjusted to 4.3, maximum flocculation of the colloids takes place. The flocculation can be produced by the addition of appropriate quantities of bentonite, a colloidal clay, the particles of which are negatively charged. The action is one of mutual flocculation of oppositely charged colloids, and results in a brilliantly clear honey which is lighter than the original in colour.

W. P. S.

**Organic Acids in Honey.** E. K. Nelson and H. H. Mottern. (*Ind. Eng. Chem.*, 1931, 23, 335–336.)—The quantity of total volatile acids in fifteen samples of different honeys varied from 0.011 to 0.051 per cent., and consisted mainly of a mixture of formic and acetic acids. Sage honey contained the largest amount of acetic acid (0.046 per cent.) and tulip honey the largest amount of formic acid (0.024 per cent.). Citric acid and malic acid were present in all the samples, and succinic acid was detected in the samples having a relative high acidity, namely, sourwood, cotton and tulip honeys.

W. P. S.

**Detection, Determination and Occurrence of Butyric Acid in Food-stuffs.** J. Grossfeld and F. Battay. (*Z. Unters. Lebensm.*, 1931, 61, 129–161.)—Butyric acid in the presence of small amounts of acetic acid may be detected by the odour of the solution obtained on distillation, after the removal of other substances by the action of alkaline potassium permanganate and reduction of the latter in turn by means of acid ferrous sulphate (*vide infra*). The limiting amounts detectable are 1:12,500 in pure aqueous solution, and 1:10,800 in admixture with 0.6 per cent. of acetic acid. Butyric acid may be determined by the method described in detail for wine (see following abstract), the conditions for which have been established as a result of a theoretical consideration of the distribution-coefficient of butyric and caproic acids between water and xylene or petroleum spirit, and of the volatilities of the different fatty acids on distillation. The method depends on the separation of capric acid and higher fatty acids by extraction with petroleum spirit, controlled oxidation of amino acids and residual acids other than butyric and acetic acids with alkaline permanganate, followed by distillation of the butyric and some of the acetic acid, formation of their potassium salts and determination of the percentage of potassium in the residue after evaporation (*A* grms.) by the perchlorate method. Then *B* (percentage of butyric acid in the residue) =  $2.223 (141.23 - k)$ , where *k* is the perchlorate value (*cf.* Grossfeld, *ANALYST*, 1930, 55, 138), *i.e.* the percentage of potassium perchlorate from the mixed potassium salts. A table gives the corresponding values of *B* for values of *k* between 109.83 and 141.23. Then *AB*/100 gives the butyric acid in grms. It was found that for reproducible and rapid working it was sufficient to use a simple distillation apparatus under the prescribed conditions (*loc. cit.*), and to apply a correction for all losses (*cf.* Wiegner and Magasanik, *ANALYST*, 1920,

**45, 24).** The distillation-curve of butyric acid from ordinary aqueous solution indicates an almost linear relationship between the percentage in the distillate and the percentage volume distilled. This rate is less for the simple distillation of acetic acid. Distillation of 40 c.c. from 50 c.c. of solution containing all the necessary reagents, as in the prescribed conditions (*vide infra*), is rapid and reproducible, though an error is involved if the acetic acid present is in great excess over the butyric acid. The distillation curve, in the case of butter-fat, is very similar to that of pure butyric acid. For the determination of *k*, 60 per cent. perchloric acid is preferable to the usual 20 per cent., the loss by solution of potassium perchlorate being only 0.04 compared with 0.13 mgrm. per c.c. This method is preferable to titration of distillate described below. The method is applied to the following samples, for which the percentage butyric acid contents are given:—Milk (sweet 0.004, sour 0.008); cheese (Limburger 1.340, Harzer, 0.142; rindless Emmenthaler 0.066, Swiss 0.356); meat (beef 0.036, pig 0.010, mutton 0.009); sauerkraut, 0.071; pickled cucumber, 0.023 (liquor 0.022); sultanas and currants, 0. The following results are in grms. per litre:—Wine labelled "Malaga," 0.110; best Samos, 0; poor sweet wine labelled "Samos," 0.203; dry red wine, 0; (*cf.* following abstract); wine distillate, 0.087; brandy, 0.234; beer (light 0.048, dark 0.081); pressed yeast, 0.019. J. G.

**Adulteration of Sweet Wine and its Detection by Determination of the Lower Fatty Acids (Butyric Acid).** A. Miermeister and F. Battay. (*Z. Unters. Lebensm.*, 1931, 61, 161–171.)—As a qualitative test for carob (St. John's bread) in currant or raisin wine, 50 c.c. of wine are evaporated to 10 c.c. on the water-bath with 1 c.c. of 50 per cent. potassium hydroxide solution, and the residue acidified with 25 per cent. phosphoric acid and diluted to 200 c.c. From this 50 c.c. are then distilled, 5 c.c. of an alkaline 1 per cent. solution of potassium permanganate added to the distillate and, after 2 hours, 5 c.c. of acid ferrous sulphate solution (*vide infra*), and the mixture redistilled. If 1, 2 and 7 c.c. fractions are collected, the carob odour (*iso*-butyric acid) can be detected in the 2 c.c. fraction. If the test is positive, butyric acid should be determined by the method of Grossfeld and Battay (*cf.* preceding abstract). The wine (200 c.c.) is distilled with phosphoric acid, 160 c.c. collected, neutralised with *N* potassium hydroxide solution, 4 c.c. of a 50 per cent. potassium hydroxide solution added in excess, and the mixture heated for 30 minutes under a reflux condenser. After acidification with phosphoric acid the solution is diluted to 200 c.c., and 160 c.c. shaken with 40 c.c. of petroleum spirit (b.pt. 70° C.) for 2 minutes. The aqueous phase is neutralised with *N* sodium hydroxide solution, an excess of 1 c.c. added, the liquid evaporated, and the residue extracted with 10 c.c. of water. Potassium permanganate (20 c.c. of a 1 per cent. solution) is added, followed after 24 hours by 20 c.c. of a mixture containing 200 grms. of ferrous sulphate and 50 c.c. of sulphuric acid per litre, to destroy any excess. The mixture is then distilled (*cf.* preceding abstract), 40 c.c. collected in a 100 c.c. flask (previously dried at 120° C. and weighed), and titrated exactly to the end-point (*X* c.c.) with 0.1 *N* potassium hydroxide solution in the

presence of 3 drops of 0.25 per cent. phenolphthalein solution. Then  $0.013856X$  gives the potassium perchlorate equivalent ( $C$ ). The liquid is then evaporated, the residue dried at  $105^{\circ}$  to  $140^{\circ}$  C. and weighed ( $A$ ), when  $k=100C/A$ . In accurate work  $k$  may be determined from the weight of potassium perchlorate produced by treatment with perchloric acid (*loc. cit.*). The butyric acid content is thence found as described, and corrected (by the factor 1.15) for extraction, distillation and other losses. The mean molecular weight of the "middle" fatty-acids (which proved to be absent from sweet wines) may be found by washing the petroleum spirit extract with 20 c.c. of water, followed by 10 c.c. of 0.1  $N$  potassium hydroxide solution, and by 35 c.c. of water. The alkaline extract is distilled with 5 c.c. of 25 per cent. phosphoric acid, and the distillate treated as already described, to determine  $k$ . No butyric acid was obtained from currants or raisins or from genuine sweet wines made from them. Of 13 purchased samples of Samos or Grecian sweet dessert wines, 2 contained no butyric acid, 5 contained 30 to 60 mgrms. per litre, and 6 (having distinct carob character) 80 to 250 mgrms. per litre. The ultra-violet lamp, which is considered useful only for sorting purposes (*e.g.* when the wine contains more than about 35 per cent. of carob), gives an intense luminescence with carob wine, but only a feeble colour with pure raisin wine. The fluorescence was simulated by ethyl butyrate, but not by butyric acid or by *iso*-butyric acid or its ethyl ester (*cf.* Berg and Stockert, *id.*, 1929, 57, 448; Kickton and Berg, *id.*, 1928, 56, 397). J. G.

**Detection of Fruit Wine in Grape Wine by the Sorbitol Method.** M. Klostermann and W. Fachmann. (*Z. Unters. Lebensm.*, 1931, 61, 100–103.)—The wine (100 c.c.) is treated with 5 grms. of animal charcoal, filtered into a 300 c.c. flask, and evaporated on the water-bath under reduced pressure till viscous. Dextrin and pectins are then eliminated by extraction with 150 c.c. of absolute methyl alcohol, and amino and organic acids precipitated in the extract by 1 c.c. of lead acetate, the precipitate being collected on a porcelain suction-filter and washed with methyl alcohol. Excess of lead is removed from the filtrate by passage of a minimum volume of hydrogen sulphide, and the filtered solution evaporated under reduced pressure to 20 c.c. in a large test-tube provided with a capillary air-inlet to prevent foaming. Three c.c. of pyridine and 3 times the volume of acetic anhydride are then added, and, after 45 minutes on the water-bath, 20 c.c. of water are added, the acidity reduced by addition of sodium carbonate (the solution saturated with sodium chloride, and the yellow oil which separates extracted with ether. The ethereal extract is washed and evaporated, the residual acetyl sorbitol dissolved in warm water, and the solution cooled in ice, when it yields crystals (m.pt.  $98$  to  $99^{\circ}$  C.). The procedure, which may be made more sensitive by the use of Jahr's method (*ANALYST*, 1930, 55, 452), will detect additions of 2.5 per cent. of fruit wine, and is preferable to the production of benzylidene sorbitol (Werder, *id.*, 1929, 54, 476). J. G.

**Determination of Trigonelline in Raw and Roasted Coffees.** F. E. Nottbohm and F. Mayer. (*Z. Unters. Lebensm.*, 1931, 61, 202–210.)—The



method is a modification of that of Lendrich and Nottbohm (*ANALYST*, 1909, **34**, 214, 484). The sample (20 grms.) is extracted with chloroform, which removes more extraneous matter than carbon tetrachloride, the solvent removed completely, and the residue extracted 3 times, at hourly intervals, with 96 per cent. alcohol. The alcoholic liquor is mixed with excess of lead acetate solution (strength not stated), and the orange precipitate (yellow in dilute solutions) is filtered off on a large folded paper, washed with alcohol, and the lead removed from the filtrate by means of hydrogen sulphide. The resulting filtrate is evaporated to 50 c.c. with 5 c.c. of hydrochloric acid (sp. gr. 1.124) to destroy the sugars, the solution filtered from the oily deposit, and the filtrate evaporated with a little charcoal and again with water and a few drops of acid. The residue is warmed with water and more charcoal, filtered, washed with water, and the clear and colourless filtrate treated with 3 drops of hydrochloric acid and 11 c.c. of 0.1 *N* iodine solution. A cloudy brown precipitate results, which settles out in crystals after 10 minutes, when it is filtered on an asbestos Gooch filter, washed with a little cold water, dissolved in warm alcohol, and the diluted solution titrated with 0.1 *N* sodium thiosulphate solution (1 c.c. 0.1 *N* iodine solution  $\equiv$  4.90 mgrms. trigonelline). Raw coffee gives steel-blue, spear-shaped crystals of the iodine compound, and roasted coffee an oily modification of the same compound (containing 1 mol. of trigonelline to 3 of iodine) crystallising slowly in leaflets. After the titration the solution may be shaken with silver oxide, filtered after some time, and the filtrate evaporated with excess of hydrochloric acid and a little charcoal. The residue is extracted with 96 per cent. alcohol (which leaves the silver and sodium chlorides) and filtered hot. The compounds ( $C_7H_7NO_2$ )<sub>4</sub>, 3HCl, 3AuCl<sub>3</sub> (large yellow needles, m.pt. 186° C.) and  $C_7H_7NO_2$ , HCl, AuCl<sub>3</sub> (m.pt. 198° C., 4-sided plates or prisms) are produced by precipitation directly with gold chloride and recrystallisation from hot dilute hydrochloric acid, and by precipitation of the hydrochloride with gold trichloride in the presence of acid and recrystallisation from a hot dilute acid solution of the gold salt, respectively. The compound ( $C_7H_7NO_2$ )<sub>2</sub>.HCl.AuCl<sub>3</sub> m.pt. 248 to 249° C. (Gorter) is produced as yellow aggregates under conditions which are not easily defined. The sources, properties and formulae of the various gold chloride hydrochloric acid salts obtained by other workers are tabulated (*cf.* Lendrich and Mayer, *ANALYST*, 1931, 326).  
J. G.

**New Triglyceride obtained on Oxidising Cocoa Butter.** J. Bougault and G. Schuster. (*Compt. rend.*, 1931, **192**, 953-954).—Oxidation of cocoa butter by Hilditch's method (*ANALYST*, 1929, **54**, 243) shows that nearly all the glycerides are unsaturated, and it was found possible to isolate a palmito-stearoazelate derived from the neutral palmitostearo-olein, owing to the solubility of the sodium salt in hot water. This glyceride is crystalline, melts at 58°-59° C., and is insoluble in water, slightly soluble in 95 per cent. alcohol, soluble in boiling alcohol, and in ether, acetone, and chloroform, but only slightly so in petroleum spirit. The molecular weight, saponification value and acid value accord with its being a palmito-stearoazelate. It was found possible, by saponification, to detach the

azelaic acid from the molecule, and a neutral palmito-stearin, functioning like a free alcohol and crystallising in mica-like leaves, melting at 34° C., was isolated. At least 36 per cent. of the palmito-stearic triglyceride is present in cocoa butter.

D. G. H.

**Raw Tobacco. Nicotine Content and its Retention at Various Temperatures.** C. Pyriki and H. Dittmar. (*Z. Unters. Lebensm.*, 1931, 61, 210–217.)—The nicotine content of tobacco is dependent on the nature of the plant, the soil, the fertilisers used, climatic conditions, etc. In general, low nicotine contents are indicated by light colour and a thin leaf. The nicotine content is less in the flower than in the upper leaves (by 0.1 to 2 per cent.), and increases along the leaf from stem to tip according to the extent of exposure to sun. The stalks usually contain less than 0.5 per cent., and this accounts for the high nicotine contents of cigars from which these are removed. Pfyl and Schmitt's method (*ANALYST*, 1927, 52, 728) gave the following percentages of nicotine in loose, fermented oriental tobaccos:—Grecian (19 samples), 0.75 to 3.41; Bulgarian (7), 0.63 to 3.17; Turkish (14), 0.90 to 2.17. Determinations of nicotine and of moisture content (by distillation with toluene, Gawrilow and Ewslina, *Biochem. Z.*, 1929, 208, 79) made after 3 hours at 50° and 95° C. indicate that (particularly at 95° C.) the loss of nicotine is greatest from the moistest tobaccos. The moisture removed by the action of calcium chloride for 48 hours in a vacuum at the ordinary temperature is less than that expelled (in air) after 3 hours at 50° C.; after 3 hours at 120° C. 0.4 to 1 per cent. remains. The results also indicate that, after drying at temperatures above 95° C., low nicotine values (0.2 to 0.4 per cent.) are obtained, owing to volatilisation, but that below 95° C. the values agree to within 0.1 per cent. Other volatile basic substances were determined by steam-distillation of the tobacco and titration of 100 c.c. of the distillate with 0.1 *N* acid solution, and the results indicate that drying at a little above 50° C. produces a loss compared with desiccation over calcium chloride. In the case of a "nicotine free" tobacco examined by Petri (*Z. Unters. Lebensm.*, 1930, 60, 123) lower results were obtained, and it is pointed out that the technique of Pfyl and Schmitt (*loc. cit.*) must be followed carefully, as a small titration error is multiplied in the final nicotine content. In particular, the dipicrate should be filtered on a small paper, alkali free from carbon dioxide should be used, and the titration carried out in a micro-burette; the blank on the reagents should be deducted.

J. G.

**New Reaction for Capsaicin.** K. v. Fodor. (*Z. Unters. Lebensm.*, 1931, 61, 94–100.)—The sample is dried and powdered, and 2 grms. are shaken with 10 c.c. of dry acetone, allowed to settle for 3 hours (or centrifuged), 5 c.c. pipetted off, and 9 drops of hydrochloric acid added. This mixture is shaken gently with 0.1 grm. of ammonium vanadate. A blue colour results from 0.08 per cent. of capsaicin, varying through green to brown-green for 0.01 per cent., the limiting concentration for the test. The brown colour is probably due to oxidation of carotene and capsantin in the sample (*e.g.* capsicum), and hence provides another test, in which the brown colour (turning to light yellow) is produced by addition

of 3 drops of hydrochloric acid and 4 c.c. of 33 per cent. hydrogen peroxide to 5 c.c. of the acetone extract. For quantitative work it is preferable to prepare an extract in dry ether (shake for 45 minutes), and to add to 10 c.c. of this a 1 per cent. solution of vanadium oxytrichloride in carbon tetrachloride. This reagent, which is more stable and more sensitive than the ammonium vanadate reagent, should be added in drops till no further colour is developed, and excess avoided. The colour is stable for a week, and may be matched against a series of standards containing 0, 0.2, etc., to 0.8 c.c. of a 0.1 per cent. solution in acetone of capsaicin (extracted from paprika in alcohol). Capsaicin was absent from Spanish pepper, and 0.01 per cent. or less was found in refined Hungarian paprikas. The blue vanadyl capsaicin was prepared in bulk from the calculated quantities of pure capsaicin and vanadium oxytrichloride in carbon tetrachloride solution, washed with this solvent, and dried in a vacuum over phosphorus pentoxide at 80° C.; it was shown to have the composition  $C_{18}H_{26}NO_3 \cdot VOCl_2$ . J. G.

**Purification of Common Salt [American].** T. B. Brighton and C. M. Dice. (*Ind. Eng. Chem.*, 1931, 23, 336-339.)—The refined salt produced in the United States, particularly that from the Great Salt Lake, contains traces of substances which yield an objectionable odour when the salt is dissolved in warm water. The substances may be removed by washing the salt with brine and then heating it at a temperature not exceeding 225° C. W. P. S.

**Determination of Choline and of Acetylcholine.** L. Lematte, G. Boinot, E. Kahane, and M. Kahane. (*J. Pharm. Chim.*, 1931, 123, 371-385.)—The assay of acetylcholine involves the quantitative determination of the acetylcholine present and also its index of decomposition. The determination may be by means of either phosphotungstic or silicotungstic acid; the method is the same for choline and acetylcholine. Since these substances are appreciably soluble in the mother liquors, the reactions are carried out in as concentrated a medium as possible, and washing is reduced to a minimum. If approximately 0.1 grm. of choline is present, 15 c.c. of a 10 per cent. solution of the tungstic acid are added, and after keeping for a few minutes on a boiling water-bath, the mixture is cooled and filtered. The precipitate is washed three times by decantation, dried for 1 hour at 105° C. and weighed, and the weight, multiplied by 0.131, gives the weight of choline. If the phosphotungstate is calcined for 15 minutes at red heat the dark green anhydride is formed; and the same procedure may be used with silicotungstic acid. Acetylcholine may also be weighed as the phosphotungstate, or its anhydride, or as the silicotungstate or its anhydride. Owing to solubility the results are consistently 2 to 3 per cent. below theory. The ashing of the acetylcholine phosphotungstate gives a residue of 86.38 per cent., and the ashing of choline phosphotungstate a residue of 89.74, whilst for a mixture the figure will be intermediate. A weighed portion of the dried precipitate is calcined, cooled and weighed again, and the ratio obtained compared with tables or curves giving the corresponding index of decomposition. D. G. H.

## Biochemical.

**Tin in the Animal Organism.** G. Bertrand and V. Ciurea. (*Compt. rend.*, 1931, 192, 780-782.)—The proportions of tin present in the various organs of the body of the ox, the horse and the sheep were determined as follows:—The organs were removed from the carcase immediately after slaughtering and, after being cleaned, were heated for several days in a roomy quartz vessel with a mixture of sulphuric and nitric acids. After all the organic matter had been destroyed, the excess of acid was eliminated by evaporation in a platinum dish, the residue then treated with hydrochloric acid and the silica filtered off. After precipitation of the copper, lead, tin and platinum as sulphides by means of hydrogen sulphide, the precipitate was treated to convert the tin into stannic acid. For the various parts of the animal, the proportions of tin, in mgrms. per kilo of material, varied: for the ox, from 0.4 in the stomach to 9.48 in the skin; for the horse, from 0.65 in the stomach to 8.53 in the skin, and, for the sheep, from 0.68 in the large intestine to 6.20 in the skin. The tongue is even richer in tin, the figures for the muscle and mucus of this organ being 12.2 and 18.65 respectively for the ox, and 16.45 and 26.11 for the sheep.

T. H. P.

**Unsaponifiable Lipids of Lettuce.** I. Carotene. H. S. Oleovich and H. A. Mattill. (*J. Biol. Chem.*, 1931, 91, 105-117.)—The recent work of various investigators leaves little doubt that carotene is the plant source of the vitamin A of animal tissue. The confirmation of this relationship tends to harmonise some discordant facts and presents further interesting problems, especially the chemical reactions of carotene and the mechanism of its transformation into vitamin A. A few observations upon the properties (physical, chemical and physiological) of carotene are now recorded, and further proof of the vitamin A activity of this substance obtained from another vegetable source, lettuce, is presented. Crystalline carotene (brilliant red pleochroic crystals) was obtained from the unsaponifiable lipids of lettuce. The 200 mgrms. isolated from 10 kilos. of dried lettuce, representing 140 kilos. of fresh leaves, were probably only a small fraction of the original content. Carotene crystallises in the hexagonal system; the external forms of the crystals are many and varied, depending on several factors, but, primarily, on the solvent; e.g. rhombohedrons were obtained from petroleum spirit, triangular plates from acetone, etc. It is shown that the fading of the colour of the crystals at high temperatures is not an oxidation. Carotene solutions may be bleached by heat, ultra-violet light, and by the presence of autoxidisable fats. The presence of hydroquinone delays for a variable length of time the bleaching of carotene solutions by any of the above mechanisms. Hydroquinone protects ethyl laurate solutions of carotene apparently indefinitely. Such solutions are satisfactory for physiological studies. In autoxidisable mixtures, carotene is an active pro-oxidant; this property is unique in a hydrocarbon. The physiological activity of carotene as vitamin A is confirmed; under widely differing conditions, the growth induced by feeding rats with carotene seems to be directly proportional to the

amount given; 0.005 mgrm. permits an increment of 3 to 5 grms. in the weight of rats deprived of vitamin A. Since carotene in the solid state is decolorised and rendered inert, physiologically, by heat in the absence of oxygen, and since in solution it undergoes this change even more rapidly under the influence of heat or ultra-violet radiation, it follows that the resultant achroocartene is not a product of oxidation but rather of an intramolecular rearrangement, or possibly of polymerisation. Inasmuch as hydroquinone delays the transformation of carotene under these conditions, its function as an anti-oxidant must be extended to include the capacity to prevent the shift in electrons, if such it is, which attends the thermal or photoelectric change of the unstable to the colourless and more stable form of carotene. The pro-oxidant character of carotene, a capacity found in smaller degree in achroocartene, shows that the former possesses greater molecular energy than the latter. The electronic significance of conjugate double bonds, of which carotene possesses eleven, is, as yet, obscure. Obviously the commonly measured stability of vitamin A (carotene) in various vegetable foods exposed to heat and light does not depend upon the carotene itself or on the presence of oxygen, primarily, but is conditioned by the presence of other substances. Some properties of achroocartene are given.

P. H. P.

**Association of Vitamin A with Greenness in Plant Tissue. III. Vitamin A Content of Asparagus Grown under Light of Various Qualities. J. W. Crist and M. Dye. (*J. Biol. Chem.*, 1931, 91, 127-134.)**—Experimental evidence is presented which gives added support to the conception that in some manner or other the elaboration of vitamin A in the plant is connected with the development of the chlorophyll pigment. A brief summary is given of the conclusions reached by other workers on the subject. For the first time, a reasonably successful attempt has been made to establish this association on a quantitative basis. It was necessary to produce plants which would vary gradually in chlorophyll content from zero to a maximum quantity, and to determine their respective vitamin A efficiencies by the use of test animals. A preliminary experiment with varicoloured and uncoloured light filters gave every indication of the possibility of using such a method to secure asparagus tips of variable greenness whose vitamin A properties as food for test animals (rats) could be correlated with their degree of greenness. Accordingly, specially manufactured filters were obtained, and used in experiments with asparagus. From the results obtained it seems reasonable to conclude that, within the restrictions of the two variables expressed by some non-linear relationship, chlorophyll content is a limiting factor on vitamin A synthesis in the vegetative parts (in this case the stem tip) of the plant. This gives point to the contention of Schertz (*Science*, 1928, 68, 48) that further progress in a knowledge of vitamin A may depend much upon the industry and the success of the biochemist and the plant physiologists in their efforts to solve the mysteries of chlorophyll and its functions.

P. H. P.

**Chemistry of Vitamin B<sub>2</sub>. B. C. Guha. (*Nature*, 1931, 127, 594-595.)**—Cold aqueous extract of commercial liver extract is rich in vitamin B<sub>2</sub>, being

effective in producing good growth in young rats on a  $B_{12}$ -deficient diet, in a daily dose representing 40–60 mgrms. of the original liver extract. The vitamin is not precipitated by picric acid, benzoyl chloride, flavianic acid, or litharge, and it is not precipitated or destroyed by nitrous acid. Neutral lead acetate partially precipitates it at pH 4.6 or 7.0, and silver nitrate precipitates the bulk of the active material, but baryta does not precipitate it in either aqueous or 50 per cent. alcohol solution. Norit adsorbs the factor at the natural pH (4.6) of the aqueous liver extract, but this cannot be elutriated by acid, alkaline or neutral water-alcohol mixtures or by dilute saponin solution. Phosphotungstic acid gives an inactive precipitate and a slightly active filtrate, a combination of these two being equally unsatisfactory. Esterification with ethyl alcohol leaves the bulk of the activity in the non-esterified portion, the ester being almost inactive. Trypsin is without effect on the vitamin, which is stable to sulphur dioxide, hydrogen peroxide, and ozone. If the vitamin is a single chemical entity, it is probably not a base, an acid, or a peptide, but a neutral substance, this conclusion being supported by the results of experiments on the electro-dialysis of vitamin  $B_{12}$ . This vitamin appears to be fairly readily adsorbed by neutral precipitates, so that its partial precipitation by lead acetate and by silver nitrate is probably due to adsorption on the precipitates formed. Liver extract is potent in both vitamin  $B_{12}$  and the factor specific for pernicious anaemia, but evidence, based partly on the methods of their fractionation, indicates these to be different.

Commercial liver concentrate and commercial yeast extract (marmite) are both fairly stable to autoclaving at 124° C. at pH 9, whereas aqueous extracts made from brewer's yeast, fresh ox-liver, and ox-muscle are markedly unstable under these conditions. The stability appears to be connected with the presence of certain protective materials in a given fraction.

T. H. P.

## Bacteriological.

**Thermophilic Bacteria in Milk.** M. I. Christian. (*Nature*, 1931, 127, 558.)—The sudden and violent fluctuations in the numbers of thermophilic bacteria in milk during pasteurisation at 62.8° C. led Mudge and Thorwaldson (*Milk Dealer*, Dec., 1930, 57) to suggest that the organisms are present as dormant spores which remain ungerminated, unless the milk is subjected to the action of certain physical and chemical stimuli such as heat, cold, or the action of alkalis. Support for this view is furnished by results obtained by the author with a spore-forming organism isolated from commercial sterilised milk. If milk is heated after inoculation with the spores, germination always occurs; otherwise, germination is greatly reduced and fails in the second generation. The original spores are gradually lost by a process of dilution during subsequent cultivation, and a stable vegetative form of the organism is obtained. If a small quantity of a living vegetative culture is added to a culture of heated spores capable of germination, a number of these immediately lose their germinative power and all lose it after 24 hours. If, however, the vegetative culture is killed prior to its addition to the spores, these undergo

germination, followed by normal spore formation. Apparently the stable vegetative form, which has been found to dissociate from the sporing form, bears an inhibiting factor, which is destroyed by heat. The property of lying dormant, which is possessed by the spores of thermophilic bacteria in milk, and which is lost on heating, is possibly due to the presence of a similar inhibitory factor. T. H. P.

## Toxicological and Forensic.

**Poisoning by Methyl Alcohol.** A. Sartori. (*Chem. Ztg.*, 1931, 55, 259.)—Dissection, 6 days after death, of the body of a man who died during the early years of the War after drinking Russian spirit having indicated no definite cause of death, a mixture of portions of the various internal organs and their contents, with the blood and urine, was mixed with 20 per cent. of salt and distilled in a current of steam. A part of the distillate of about 500 c.c. (from 970 grms. of material) gave a negative result when tested for formaldehyde by boiling with milk and a hydrochloride acid solution of ferric chloride. The remainder of the distillate was filtered, and the filtrate neutralised accurately, mixed with 20 per cent. of salt and distilled to yield 170 c.c. of distillate. A few c.c. of this liquid were acidified with dilute sulphuric acid, heated with a small amount of potassium permanganate to 50° C., and filtered. When boiled with milk and a mixture of ferric chloride, the clear filtrate assumed an intense violet coloration. Another portion of the second distillate gave a distinct violet colour when mixed, with cooling, with concentrated sulphuric acid and a solution of morphine sulphate in the same acid. These reactions indicate the presence of formaldehyde in the oxidised distillate, and hence that of methyl alcohol in the body organs examined.

To test these organs for formic acid, the residual liquid remaining after the second distillation was strongly acidified with phosphoric acid and distilled. The distillate was acidified with sulphuric acid, digested for some hours with magnesium turnings, and filtered. When boiled with milk, hydrochloric acid and ferric chloride, the liquid then gave no violet colour, the absence of formic acid from the organs being thus indicated.

Similar results were recently obtained in the case of a soldier found dead in a train from Russia.

T. H. P.

**Colorimetric Determination of Traces of Cadmium in Organic Matter.** L. T. Fairhall and L. Prodan. (*J. Amer. Chem. Soc.*, 1931, 53, 1321–1323.)—The organic matter (100 grms.) is destroyed by digestion with nitric acid; after addition of sulphuric acid (10 c.c.) the oxidation is continued until white fumes are freely given off by the colourless solution. The liquid (75 c.c.) is treated with 0.5 mgrm. of copper as sulphate, 2 grms. of sodium citrate, ammonia to a hydrogen ion concentration of  $10^{-3}$  (thymol blue and bromchlor-phenol blue), hydrogen sulphide for 5 to 10 minutes, and a drop of 5 per cent. aluminium chloride solution. After standing for 6 to 12 hours, the precipitate is collected, dissolved in nitric and hydrochloric acids, and the solution evaporated to dryness. The sulphide precipitation is repeated twice more, the sodium citrate being omitted the last time,

CH adjusted to  $10^{-3}$  by means of dilute potassium hydroxide solution, and the sulphide dissolved as before. The final chloride solution is evaporated to dryness, the residue dissolved in water, made up to bulk, and an aliquot part transferred to a Nessler tube. To this tube are added 5 drops of 10 per cent. potassium cyanide solution, distilled water, and 5 c.c. of hydrogen sulphide water. The colours of the solutions are matched against those of a standard cadmium solution in the light of a quartz mercury vapour lamp. The ultra-violet rays produce an intensification of the yellow colour of the sulphide, 0.01 mgrm. of cadmium in 50 c.c. producing a perceptible difference in tint. An accuracy of 4 per cent. is claimed for contents of 0.4 to 1 mgrm. of cadmium in 100 grms. of organic material. The reagents used should be free from lead. Dark or turbid solutions indicate that iron has not been completely removed. The standard tubes should be prepared at the same time as those containing the solution under investigation, and should not be allowed to stand overnight.

W. R. S.

## Organic Analysis.

**Effect of Light on the Determination of Ethylene.** J. L. Oberseider and J. H. Boyd. (*Ind. Eng. Chem., Anal. Edition*, 1931, 3, 123.)—Ethylene can be separated sharply from saturated paraffin hydrocarbons by absorption in one-third saturated bromine water in a Williams pipette, provided that the apparatus is shielded from direct sunlight and the glass is painted black. In direct sunlight saturated paraffin hydrocarbons are slowly absorbed by the reagent.

S. G. C.

**Use of Buffers in the Determination of Colours (Dyes) by means of Titanium Trichloride II.** O. L. Evenson and R. H. Nagel. (*Ind. Eng. Chem., Anal. Edition*, 1931, 3, 167–169.)—A method is given for the evaluation of the colouring matter in water-soluble coal-tar food colours which are on the permitted list of the United States Department of Agriculture. It is based on Knecht and Hibbert's method, and is as follows: In the case of triphenylmethane dyes, the solution (200 c.c.) of 30 grms. of the buffer salt required, as noted below, is heated to boiling, and cooled to 85° C. under carbon dioxide; 1 grm. of the dye dissolved in 100 c.c. of water is added, and the solution is titrated with standardised titanous chloride at 60° to 70° C. For other dyes, 10 to 20 grms. of the buffer salt are used, and the volume of the solution should be approximately 100 c.c. at the start of the titration, which is carried out with the solution hot under carbon dioxide, and stirred mechanically. A table is given in the paper showing the most suitable buffering agents for particular dyes. Sodium bitartrate, Rochelle salt, or sodium tartrate is recommended for Orange I (150), Ponceau SX, Tartrazine (640), Naphthol Yellow (10), Indigotine (1180), Brilliant Blue FCF, Fast Green FCF, Light Green SF Yellowish (670), Guinea Green B; and sodium citrate for Amaranth (184), Ponceau 3R (80), Sunset Yellow FCF. The numbers in parentheses following the name of each dye are those given in the 1924 edition of the Colour Index.

S. G. C.



**New Higher Alcohols produced during the Hydrogenation of Fish Oils.** S. Ueno and R. Yamasaki. (*J. Soc. Chem. Ind., Japan*, 1931, **34**, 35B.)—One kilo. of unsaponifiable matter from a fish oil was washed with methyl alcohol in order to separate the oxy-compounds from the hydrocarbons. The portion soluble in methyl alcohol was freed from the minute amounts of malodorous aldehydes by means of sodium bisulphite, washed with water, and dried over anhydrous sodium sulphate, yielding 20 grms. of higher alcohols. These crude oils were distilled at 15 mm. pressure and collected in six fractions ranging from 90–100° C. to 150–163° C. in b.pt. From the ultimate analysis, molecular weight and iodine value, the empirical formula for the first fraction is given as  $C_9H_{18}O$ ; and for the last fraction  $C_{14}H_{30}O$ . The m.pts. of the alcohol and of the acid produced by oxidation with permanganate are too low to correspond with the primary alcohol, and it is assumed that fraction 6 is, therefore, an aliphatic iso-primary alcohol.

R. F. I.

**Quantitative Determination of Mixtures of Isomeric Unsaturated Compounds. A Review of the Iodimetric Methods and a New Bromimetric Method.** R. P. Linstead and J. T. W. Mann. (*J. Chem. Soc.*, 1931, 723–725.)—The original iodimetric method for acids (*J. Chem. Soc.*, 1927, 2565) has been found in practice to be very successful for the monobasic series, but the high reactivity of the  $\beta\gamma$  acids towards iodine diminishes with the entry of negative groups. All the  $\alpha\beta$  acids so far examined have iodine additions of 0 to 1 per cent. by the standard method, and this provides a valuable test of their purity. The estimation of mixtures of itaconic and mesaconic acids presents difficulties, since the addition of iodine under the standard conditions was negligible, but addition of bromine in most solvents was too rapid for convenient measurement. The method adopted is to standardise a 0.05 *N* solution of bromine in concentrated aqueous potassium bromide (400 grms. to 1 litre), and to run 25 c.c. quantities of this solution from the burette into 10 c.c. of water in stoppered bottles. After keeping for 10 minutes in the dark, the solutions are treated with 10 c.c. of 10 per cent. aqueous potassium iodide, and the liberated iodine titrated with 0.05 *N* thiosulphate, after which 10 c.c. of exactly *M*/15 solutions of the pure acids or their mixtures are pipetted into stoppered bottles and treated with an exactly equivalent amount of the bromine solution, calculated from *J* (amount required in c.c.) =  $100/3t_{25}N$ , where  $t_{25}$  is the thiosulphate titration of 25 c.c. of the bromine, and *N* is the normality of the thiosulphate. The back titration is carried out as for the blank. The amount of addition to the pure acids varied with temperature, from 37.3 for mesaconic and 67.3 for itaconic at 16.8° C., to 45.9 and 75.7 at 23.2° C., but the temperature coefficient is the same in each reaction, so that a reference curve was constructed and enabled a simple temperature correction to be made. For mixtures of mesaconic and itaconic acids containing 100 per cent. mesaconic acid (at 16.8° C.) the percentage addition is 37.3; 80 per cent., 43.3; 60, 49.5; 40, 55.9; 20, 62.2; and 0, 67.3. The figures obtained for the compositions of mixtures were found to be accurate to  $\pm 1$  per cent., and agreed with those determined by actual isolation of the itaconic acid.

D. G. H.

**Preparation and Properties of Highly Purified Oleic Acid.** J. H. Skellon. (*J. Soc. Chem. Ind.*, 1931, 50, 131-134T.)—Two modified processes of existing methods for the preparation of pure oleic acid are given. The liquid acids are separated from the crude acids of olive oil by a modification of Twitchell's lead salt and alcohol process by adding a solution of 200 grms. of potassium hydroxide in 500 c.c. of water to a mixture of 1 kilo. of olive oil with 2 litres of absolute alcohol, and, after refluxing for 3 hours, removing the excess of alcohol by distillation under reduced pressure, boiling the syrup with 10 per cent. hydrochloric acid, and pouring the free acids into water. Mineral acid is removed by washing, and the mixture is dissolved in 4 times its weight of ethyl alcohol and heated to boiling, after which sufficient lead acetate (dissolved in an equal volume of boiling alcohol) to combine with 24 per cent. of the total acids is added, and after standing for 12 hours the lead salts are filtered off, redissolved in hot alcohol with a few drops of acetic acid, boiled, cooled and filtered. Alcohol is removed from the filtrates, and the acids, liberated from the syrup by 10 per cent. hydrochloric acid, are extracted with ether. I. The liquid acids, amounting to 60 per cent. of the crude acids, are dissolved in 12 times their weight of a mixture in equal volumes of dry benzene and absolute alcohol, and sufficient barium hydroxide is added to the boiling solution for neutralisation to phenolphthalein, and a small amount of hot water. After rapid cooling, the oleate is collected, dried, recrystallised from 3 times its weight of moist benzene and absolute alcohol, the purified oleate suspended in warm water and decomposed by repeated shaking with 10 per cent. hydrochloric acid. On cooling, the upper layer of oleic acid is extracted with ether, dried, the solvent removed, and the oleic acid finally dried *in vacuo*, after which it is converted into the methyl ester. This is distilled under reduced pressure, and the main fraction (97 per cent. of the esters) saponified, and the acids recovered. The iodine value of the final, almost colourless, product is 90.5-91. II. The liquid acids are dissolved as in I; lithium hydroxide (about 20 per cent. of the weight of the acids) is dissolved in an equal volume of boiling water, and this is gradually added to the boiling solution of the acids under a reflux condenser, until they are neutral to phenolphthalein. On cooling, the oleate is filtered off, washed, dried, and recrystallised twice from four times its weight of alcohol, decomposed by 10 per cent. hydrochloric acid, and the recovered oleic acid further purified by conversion into, and distillation of the methyl ester. The iodine value of the final product is 90 to 90.8. The refractive index,  $n_D^{20}$ , for highly purified oleic acid is taken as 1.4610, falling slightly after 2 years; the setting point as 11.8° to 12.2° C., falling about 1° C. after a year, and the m.p. as 13°-14° C. D. G. H.

**Rosinduline as Oxidation-Reduction Indicator.** L. Michaelis. (*J. Biol. Chem.*, 1931, 91, 369-372.)—Rosinduline is recommended as an indicator for oxidation-reduction potential in a very negative potential range. Its normal potential at 30° C., referred to the normal hydrogen electrode, is

pH ..	..	..	5	6	7	8	9	10	11
Normal potential, volts	-0.161	-0.221	-0.281	-0.340	-0.395	-0.438	-0.480		

The dye, Rosinduline 2G, is the sodium salt of a monosulphonic acid of a compound called Rosindon. The dye has an intense scarlet red colour; the leuco-dye in higher concentrations is slightly yellow-brown, but colourless in the lower concentrations in which the dye may be used as an indicator. The dye is readily soluble and stable, both in the oxidised and reduced state, even in very strongly alkaline solution, and so differs, advantageously it is thought, from all dyes of a comparable negative potential range; its potential range is even more negative than is that of any others which are recommended. The potentials are perfectly reproducible and steady, even at very high  $pH$ , so that this dye may be used for class experiments for reversible oxidation-reduction titration curves. P. H. P.

## Inorganic Analysis.

**Detection of Cadmium.** J. S. Pierce and W. T. Forsee. (*Ind. Eng. Chem., Anal. Edition*, 1931, 3, 188.)—Boiling the solution with nickel powder, as described below, is advocated for removing copper, lead, mercuric mercury, and bismuth prior to testing qualitatively for cadmium by hydrogen sulphide. To the original solution, neutral or slightly acid, and containing an excess of sulphate ions but no acetate, is added 1 to 5 grms. of fine nickel powder (100 mesh or finer), and the whole boiled for from 3 to 5 minutes. The liquid is filtered. A portion of the filtrate is brought to an acidity of 2  $N$  in acetic acid, and hydrogen sulphide passed into it for 15 seconds, when the appearance of a yellow precipitate indicates the presence of cadmium. If the precipitate is dark coloured, indicating incomplete removal of the other metals, the remaining portion of the solution is treated with more nickel powder. The presence of the sulphate is required to precipitate the lead as sulphate, because lead is not readily removed by nickel powder. Cadmium is partially precipitated by the nickel powder, but enough remains in the solution to respond to the test with hydrogen sulphide. S. G. C.

**Structure of Ferric Thiocyanate.** H. I. Schlesinger and H. B. Van Valkenburgh. (*J. Amer. Chem. Soc.*, 1931, 53, 1212–1216.)—The red colour produced by the addition of thiocyanate to ferric salt is usually ascribed to ferric thiocyanate. The compound is soluble in ether, which extracts it from water; the ethereal solution does not contain alkali, which proves that the coloured substance is not  $M'_2[Fe(CNS)_6]$ . When the aqueous red solution is electrolysed, the red colour migrates toward the anode, while ferric ion is found at the cathode; the complex nature of the compound is further borne out by the molecular weight, which, in non-aqueous solution, is practically twice that of  $Fe(CNS)_3$ . The authors conclude that the formula  $Fe[Fe(CNS)_6]$  should be ascribed to the ether-soluble substance. W. R. S.

**Determination of Thiosulphate by Means of Ceric Sulphate.** N. H. Furman and J. H. Wallace, Jr. (*J. Amer. Chem. Soc.*, 1931, 53, 1283–1288.)—The thiosulphate solution (0.5 to 0.7 gm. of salt) is treated with 0.3 to 0.4 gm. of potassium iodide and starch solution, diluted to 250 c.c., and titrated with 0.1  $N$  ceric sulphate solution. The results are stoichiometric. W. R. S.

**Separation of Calcium and Magnesium by the Molybdate Method.**

**R. C. Wiley.** (*Ind. Eng. Chem., Anal. Edition*, 1931, 3, 127-129.)—To the boiling, concentrated, neutral or slightly ammoniacal solution containing the calcium and magnesium is added slightly ammoniacal ammonium molybdate solution (about 0.4 *N*), at the rate of about one drop per second, until an excess is present. The boiling is continued until the precipitate of calcium molybdate has settled (about 10 minutes' boiling is required), the solution is kept until cold (about 45 minutes); the precipitate is filtered off on a weighed Gooch crucible, with the aid of gentle suction, and washed 10 times with 10 c.c. of hot water. The crucible is heated at 130° C. for 30 minutes and then ignited before weighing. The factor for converting calcium molybdate into calcium is 0.2002. The magnesium in the filtrate is determined as magnesium pyrophosphate with, however, only a single precipitation of the magnesium ammonium phosphate. Good results were obtained in test experiments with mixed solutions of calcium chloride and magnesium nitrate, with and without the addition of several grms. of ammonium chloride. A large excess of ammonium molybdate should be avoided in the precipitation; before the filtration, a drop of the liquid should be tested with a saturated solution of pyrogallol in chloroform, when a brown colour indicates an excess of molybdate.

S. G. C.

**Determination of Lanthanum (New Colorimetric Method). I. M.**

**Kolthoff and R. Elmquist.** (*J. Amer. Chem. Soc.*, 1931, 53, 1217-1225, 1225-1232, 1232-1236.)—The determination of lanthanum after precipitation as oxalate and as hydroxide was studied by precision methods. The solubility of the two compounds was investigated with the help of a new colorimetric method based on the bright violet coloration given by sodium alizarinate in solutions containing as little as 0.1 mgrm. of lanthanum per litre in acetate solution. The filtrates from the oxalate precipitates were evaporated with excess of sulphuric acid to complete dryness, and the residue taken up in one c.c. of a solution of ammonium acetate and acetic acid (both 2 *N*) and 10 c.c. of water, and the reagent (0.4 c.c. of 0.1 per cent. sodium alizarinate solution) added; a solution of known lanthanum content was used as the standard. The coloured compound flocculates after some hours. The method can be used for concentrations between 0.1 and 2 mgrms. per litre. The solubility of the oxalate was found to be 2.08 mgrms. of anhydrous salt, that of the hydroxide 0.7 mgrm. of  $\text{La}_2\text{O}_3$ , per litre at 25° C.

**Oxalate precipitation.**—The determination can be made by the volumetric method, either the excess oxalic acid in the filtrate, or the solution of the washed precipitate in warm dilute sulphuric acid being titrated. It is, however, indispensable to wash the precipitate very thoroughly with water so that the adsorbed oxalic acid may be completely removed. A large excess of precipitant is required for quantitative precipitation, e.g. 50 c.c. of *N* oxalic acid for 0.15 gm. of  $\text{La}_2\text{O}_3$ . After standing overnight the precipitate is collected and washed with cold water until methyl orange gives with the washings the same colour as it does with the wash

water, then with about 175 c.c. more water (about 275 c.c. in all). The washed precipitate is dissolved in warm dilute sulphuric acid and the solution titrated with permanganate. Accuracy within 0.2 per cent. is claimed. Neither for the volumetric nor for the gravimetric method should alkali oxalate be used, as there is considerable co-precipitation, especially of the potassium salt. The authors' investigations prove that this is due to double-salt formation. For gravimetric work, the oxalic acid solution in excess (*vide supra*) is added, with constant stirring. The precipitate is filtered off the next day and ignited; drying yields no compound of constant weight at any stage of the operation. The ignition must be continued for several hours at 850° C. in an electric muffle, *i.e.* till constant weight is attained (*cf.* ANALYST, 1930, 55, 650). The results of test experiments were consistently high by 0.15 per cent.; the authors express preference for the volumetric method.

*Hydroxide precipitation.*—Lanthanum can be determined volumetrically by precipitation as hydroxide and measurement of the amount of standard acid required to dissolve the precipitate. If the alkali—sodium or ammonium hydroxide—is added to the lanthanum solution, the results will be low, as the precipitate contains basic chloride. If, however, the lanthanum solution is slowly added to a five-fold excess of alkali, the results closely agree with the calculated values. The precipitate is collected the next day, washed with 50 per cent. alcohol till free from ammonia (phenol red test), dissolved in excess of standard acid, and the excess determined with standard alkali and methyl orange. Alternatively, a gravimetric process may be used: the hydroxide precipitate, produced and collected as described, is washed with dilute ammonia, ignited to constant weight as before, and weighed in a closed weighing bottle. This precaution must always be adopted when lanthana is weighed, as it attracts moisture and carbon dioxide. The oxide obtained by ignition of the oxalate or hydroxide to constant weight at 850° C., when subjected to prolonged ignition at a white heat, shows marked increase in weight (up to about 20 per cent.). The authors ascribe this to the formation of higher oxides.

W. R. S.

## Microchemical.

**A System for the Microchemical Identification of Alkaloids.** J. F. H. Amelink. (*Pharm. Weekbl.*, 1931, 68, 159–185.)—The system for the identification of 78 of the alkaloids utilises the formation of crystalline reaction products or precipitates, with one or more of 8 reagents, the reagents being chosen so that the reaction of each alkaloid with the 8 reagents is unlike the others. A complete table of the results of the action of each of the 8 reagents on the 78 alkaloids is given, together with 203 drawings of the microscopic appearance of the various crystalline products formed.

The reagents used are:—(1) A 10 per cent. platinic chloride solution, both in neutral solution and in dilute hydrochloric acid solution (0.5 *N*). Sometimes sodium iodide is added with a little platinic chloride solution, when the iodoplatinate is formed, which is usually less soluble than the chloroplatinate. (2) A 5 per

cent. gold chloride solution, used both in neutral and in dilute hydrochloric acid solution (0.5 N). Gold bromide is also sometimes used, as the bromo-aurates are usually less soluble than the chlorides. (3) Mercuric chloride, either in neutral or in dilute hydrochloric acid solution (0.5 N), when double salts may be formed. (4) Potassium ferrocyanide in dilute hydrochloric acid solution (0.5 N). This reagent is used warm. (5) Potassium ferricyanide in dilute hydrochloric acid solution. A large excess is always used. (6) Dragendorff's reagent, which is prepared by heating 18 c.c. of water, 3 c.c. of 4 N hydrochloric acid and 7 grms. of potassium iodide, and slowly adding 1.5 gm. of basic bismuth nitrate to the boiling solution. On cooling, 1.5 gm. of iodine is added, and the solution diluted with an equal volume of water, in which condition the solution will keep. The reagent is used in two different strengths, one concentrated, diluted with an equal volume of water, and the other very dilute. (7) A 50 per cent. solution of potassium hydroxide. (8) Picrolonic acid (dinitro-methyl-phenyl-pyrazolon), used as a fine crystalline solid; it is added to the neutral solution of the alkaloid. J. W. B.

**Microchemical Identification of Harmine and Harmaline. J. F. H. Amelink.** (*Pharm. Weekbl.*, 1931, 68, 221-229.)—The properties and reactions of Merck's preparations, "Harmine" and "Harmaline," are described. "Harmine" is identical with banisterine hydrochloride,  $C_{13}H_{11}ON_2HCl \cdot 2H_2O$ . The alkaloid melts at 263° C. without decomposing. Harmine (the hydrochloric acid salt) is a white crystalline powder; the solubility in water at 20° C. is 1:40 and the solution is slightly fluorescent. With sulphuric acid the solution is yellow with a green fluorescence. With Fröhde's reagent it gives a yellow-brown colour, turning green on heating; with Erdmann's reagent a green colour, turning blue-green on warming; with Marquis' reagent a red-brown colour, turning violet on warming; and with Wasicky's reagent a yellow-brown colour which disappears on heating, and, on standing, becomes red-brown again, then red, and, finally, purple. When 0.05 mgrm. of harmine per gm. weight of the animal to be tested is injected intravenously, the first result is violent trembling, followed by cramp and death. Harmaline gives the same result when 0.005 mgrm. per gm. body weight is administered. The alkaloids can thus be identified physiologically. Chemical identification is carried out, using the 8 reagents described in the previous paper (abstract above). Harmaline, which is dihydro-harmine, melts (without decomposition) at 238° C. Merck's preparation is the hydrochloric acid salt. It is a yellow crystalline powder, yielding a yellow aqueous solution with a blue-green fluorescence, visible at a dilution of 1:1,000,000; this is a specific reaction for harmaline. When the alkaloid is heated with acetaldehyde on the water-bath a blood-red fleck is formed, soluble in chloroform or 90 per cent. alcohol, but not in ether; this can also be used as a test for acetaldehyde. Harmaline gives with concentrated sulphuric acid an intense yellow colour, turning light yellow on heating; with Fröhde's reagent, a brown-yellow colour, turning yellow-green on heating; with Erdmann's reagent, a brown-yellow colour, turning orange-yellow on heating; with Marquis' reagent, a red-brown colour becoming darker, and changing to green

on warming; with Wasicky's reagent, a yellow-brown colour, turning red-brown on heating. The reactions with the 8 reagents (see previous abstract) are carried out with either a neutral 1 per cent. solution or a solution in 0.5 *N* hydrochloric acid. Details of the results of the tests are given in the original, together with photomicrographs of the crystalline reaction products. J. W. B.

## Physical Methods, Apparatus, etc.

**Use of Tungsten Arc Lamps for Photomicrography.** E. E. Jelley. (*Nature*, 1931, 127, 200–201.)—Owing to their uniform intrinsic brilliancy and compactness, these lamps are extensively used as illuminants for photomicrography, but it is not generally recognised that the light which leaves the metal surfaces at almost tangential angles is so strongly plane-polarised that it is difficult to obtain uniform illumination when crystals are being photographed by plane-polarised light. This lack of uniformity, which is not observed with either a carbon arc lamp or the sun, may give rise to appreciable errors if a tungsten arc lamp is used for spectrophotometry without the interposition of ground glass to form a secondary source. T. H. P.

**Errata.**—P. 334, l. 4, for " $\text{ClO}_3$ " read " $\text{KClO}_3$ ."

l. 5 from bottom, for "determined from the data . . ." read  
"determined. From the data . . ."

## Reviews.

**BELL'S SALE OF FOOD AND DRUGS ACTS.** By R. A. ROBINSON. Eighth edition. Pp. xxvi, 299, and (Index) 34. London: Butterworth & Co. (Publishers), Ltd., Bell Yard, Temple Bar; Shaw & Sons, Ltd., 7–9, Fetter Lane, E.C. 1931. Price 16s. net.

The first edition of "Bell" was noticed in *THE ANALYST* of 1886, and the reviewer has proved himself to be an excellent prophet, for he wrote (p. 139): "It will doubtless have a large sale among all officers engaged in carrying out the provisions of the Acts." To those who have been familiar with this work in its later editions, this latest, the eighth, will appear almost as a stranger. It is, however, a handsome stranger, and becomes more friendly on extended acquaintance, in spite of the fact that it does not fit well into a bookcase along with its forerunners.

The present edition is edited by Mr. R. A. Robinson, who is well known as the chief officer of the Public Control Department of the Middlesex County Council, and also as the author of the notes on adulteration which were a useful feature of previous editions. Mr. Robinson's reputation is such that the book is approached with pleasurable anticipation, an anticipation which is thoroughly realised by its subsequent perusal. In a standard work, such as this, praise seems no longer to be necessary, and it follows that any adverse criticism will become more prominent than its importance warrants. The points mentioned below, therefore, may be taken merely as suggestions for consideration by the editor for future editions.

In an introduction which is a valuable new feature of this edition, as compared with the last, although there was one in the sixth edition, the duties of Food and Drugs Authorities are well set out and carefully distinguished from those of Sanitary Authorities. The Milk and Dairies (Consolidation) Act, 1925, which was in the appendix of the last edition, now follows on immediately, and the Merchandise Marks Act of 1926 and the Sale of Food (Weights and Measures) Act, 1926, are also included.

The real meaning of the regulations referring to the labelling of condensed skimmed milk is clearly set out. There seems to be an impression in certain quarters that this refers to ordinary skimmed milk. The case of *Lamy v. Watson* under the Merchandise Marks Act is not mentioned. This is the case which decided that no trader or body of traders could set up a standard for any food, unless it was the ordinary basis of contract between the buyer and seller. The reviewer has not been able to trace an official report of this case.

Section 27 (5) of the Food and Drugs Act enacts that the name of the prosecutor shall appear on the summons. It has been held by several benches of magistrates that the mere appearance of the name is not sufficient, but that it must be preceded by some such words as "The name of the prosecutor is . . ." It would be an advantage if this point were stressed.

As was recently pointed out in *THE ANALYST* (1930, 55, 40), the note on *Bakewell v. Davis* is not correct. In this case, as the Public Analyst had supervised the essential parts of the analysis, it was held that this was equivalent to the Analyst doing the analysis himself. This case, therefore, gives no support to the suggestion that a Public Analyst may rely entirely on the work of his assistants.

The suggested form for milk certificates, set out on page 182, does not appear to be the best; it might be advisable to give alternative suggestions.

The bibliography of official publications, given in Appendix I, is useful, and might be extended by the addition of the publications of the Food Investigation Board and the Empire Marketing Board, many of which are likely to be of interest to Public Analysts and Sanitary Officers.

The chemical notes might be extended with advantage. No mention is made of the adulteration of almonds with arachis nuts, or of arrowroot and cornflour with sweet potato, the sale of butter and margarine mixtures, the judgment of the



Staffordshire Stipendiary with regard to rice flour in shredded suet, etc. The remarks about glucose in the article on golden syrup (p. 292) are likely to be misunderstood by non-chemical readers and should be revised.

As already stated, these are only minor suggestions for the next edition. The book has for years been indispensable to all those whose work brings them into touch with the Food and Drugs Acts, and this latest edition will ably carry on the tradition. It is well bound and legibly printed on good paper. Typographical errors appear to be very few.

G. D. ELSDON.

HANDBUCH DER BIOLOGISCHEN ARBEITSMETHODEN. LIEFERUNG No. 308. By Prof. Dr. EMIL ABDERHALDEN. Section A, Part II, Subsection 5, containing: Die Methoden der Ligninforschung, by Walter Fuchs; and Die Terpene, by Konrad Bournot. Pp. 877-1068. Berlin and Vienna: Urban und Schwarzenberg. Price 10 gold marks.

Abderhalden's Handbuch covers anything from Chemistry to Psychology. It is obviously a very useful, but, at the same time, a highly expensive work. It is very hard to see how any one specialist could be interested in all that is to appear and already has appeared in it, and most subscribers find themselves by now in the position of the reviewer, who had to cancel his subscription. The age of the encyclopaedists is past and gone. Lieferung 308 may serve as a good illustration of the reviewer's contentions. It is written for highly specialised work, and it is most unlikely that one and the same research chemist could be interested in two such different subjects as lignin and the terpenes. Similar mixtures are to be found in most of the other 307 parts which have appeared.

The section on lignin is particularly well written, and Dr. Fuchs has shown great discrimination in selecting his material. Lignin has been the object of many recent investigators, and Freudenberg's results are well known to disagree with those of other workers. Fuchs, therefore, has done a good service to chemistry by balancing, in a critical manner, the divergencies of opinion between Freudenberg and his opponents. His description of the methods of investigating lignin is, therefore, a very valuable contribution to the literature.

Section 2, which deals with the terpenes, shows very little originality. It is nothing more than a compilation of facts which are accessible in most textbooks of Organic Chemistry. No specialist would find it a help in his work, and the general chemist is scarcely interested enough to require 130 pages on the subject.

M. NIERENSTEIN.

TEXTBOOK OF QUANTITATIVE ANALYSIS. By W. T. HALL. Pp. vii+279, with 42 illustrations. London: Chapman & Hall. 1930. Price 12s. 6d. net.

Many chemists throughout the world are familiar with "Treadwell and Hall" as one of the most reliable works on general analytical methods, although it was

originally produced as a students' textbook. The suggestion had been made that it should again be modified to suit its original purpose, but, instead, an entirely new volume, under the above title, has been produced by the translator of the original work.

The volume is based on a course in analytical chemistry provided at the Massachusetts Institute of Technology; and, although the methods described are almost identical with those in the older work, it is obvious that this textbook is far from being a mere abridgment of its predecessor. The subject-matter includes the usual requirements of the student, such as the use and calibration of apparatus, calculations, volumetric neutralisation, oxidation and precipitation methods. These are followed by the principles of gravimetric analysis and numerous applications of them, electrolytic analysis and a few more specialised methods, such as the estimation of carbon and nickel in steel and of tungsten and titanium. The last chapter is devoted to potentiometric titrations using hydrogen and quinhydrone electrodes, and the volume ends with a number of tables providing data required in calculations, etc. At the end of each chapter a series of "Home Problems" is provided, and any student capable of working these correctly without assistance would be thoroughly conversant with the mathematics of the subject.

The methods given have been well selected and are described with such detail that the highest degree of accuracy possible may be attained, an essential which is emphasised throughout the volume. In this connection a discussion of errors in the precipitation of barium sulphate by various reagents, given on pages 170-173, is invaluable to the student, although such a feature is but rarely met with in a textbook. It is, perhaps, a matter of opinion as to how much theory should be included in an essentially practical work, but an account of the theory of electrolysis extending to nearly 12 pages and a brief exposition of the theory of logarithms appear rather out of place.

The book is a model of careful production and is almost free from errors, only one, the trifling omission of an "o" giving "Ido Starch Reaction," being detected, while the index is complete and accurate and the illustrations clear, although there is no obvious reason why that on p. 40 should be reproduced on p. 233.

The volume will serve as an excellent introduction to industrial analysis, and will undoubtedly prove acceptable to all engaged in the teaching of analytical chemistry.

T. J. WARD.

INDUSTRIAL MICROBIOLOGY. THE UTILISATION OF BACTERIA, YEASTS AND MOLDS IN INDUSTRIAL PROCESSES. By HENRY FIELD SMYTH, M.D., Dr. P. H. and WALTER LORD OBOLD, M.S. London: Baillière, Tindall & Cox. 1930. Price 27s.

This book strikes rather a new note in bacteriological literature, for it is concerned entirely with the multitudinous fermentation reactions occurring in nature

and utilised in the arts and industries. It is in fact a compilation of almost every known fermentation process.

The industry of the authors in collecting references has been prodigious; there are no fewer than 675, many of them to patent specifications. Indeed, in this respect there is perhaps a weakness, for many of the patents described are of doubtful validity, and the processes covered of doubtful commercial utility. In some cases the authors themselves recognise this, as, for instance, in Chapter III, where, after describing a number of patents for the production of butyric acid, they use the very derisory phrase: "In brief, a compost pile of indiscriminate junk has been patented."

The book suffers a little at the outset by reason of a rather curious "General Introduction," which deals very briefly with some of the physical and chemical properties and requirements of micro-organisms. In this section such important matters as hydrogen-ion concentration and anaerobiosis are each dismissed in a few lines.

Each chapter is subdivided into a great number of short sections with cross headings, and this sometimes leads to curious results. Thus, on p. 30, the section headed Citric Acid begins: "In 1892 he described a particular group . . .," the pronoun referring to an author mentioned in the preceding Section.

The treatment of various subjects is rather unequal as to length and detail; a long chapter is devoted to butyl alcohol and acetone fermentation, but the production of casein is dealt with in ten lines. The statement that "whey . . . must be a by-product industry in cheese manufacturing to pay as a source of acid" is open to question, apart from its ungrammatical construction. It can hardly be said that lactic acid production from whey is as yet a profitable operation.

The authors feel that the policy of Prohibition in the United States makes a discussion of the preparation of fermented alcoholic beverages unnecessary, and they deliberately omit any reference to this important industry.

Chapter XII contains a valuable account of glycerin production; in Chapter XIII there is a very full description of the cultural characteristics of *Bacillus acetothylicus*. Throughout the book the precise descriptions of organisms are an important feature.

There will be general agreement with the remark, referring to the use of *Salmonella* as destructors of rodents, that "the relation of epizootics in rodents to the public health of the community question (*sic*) the advisability of their general use."

Chapter XXVI, on Microbial Thermogenesis, is interesting. It is noticeable that little is really known about the spontaneous generation of heat in fermenting masses, *e.g.* in haystacks. It should be emphasised that very little reference is made to details of technique, but the value of the book lies in the precise descriptions of the various organisms best suited to specific fermentations, and, as a work

of reference in this particular field, it is undoubtedly of value to the technical bacteriologist. Anyone who wishes to know what products and by-products can be produced from any particular substance, and what organisms can be used to produce them, will find the information here.

The book concludes with a Chapter on Patent Law, contributed by J. Howard Flint, but this deals exclusively with United States Law, and is, therefore, of somewhat limited interest to British readers.

There is, naturally, a frequent use throughout the book of American turns of speech which sound strange to English ears, but, apart from the question of idiom, the English is by no means free from grammatical errors. Four misprints were noticed, one of which, "Poseudmonas" for "Pseudomonas," should not have escaped the proof reader.

R. F. HUNWICKE.

**RECENT ADVANCES IN PHYSICAL AND INORGANIC CHEMISTRY.** By ALFRED W. STEWART, D.Sc. Sixth edition. Pp. xi+388. London: Longmans, Green & Co. 1930. Price 18s. net.

The sixth edition of Prof. Stewart's well-known book is marked by the inclusion of five new chapters. A certain amount of the material which appeared in the earlier editions has been omitted, and the remainder of the text has been revised, wherever necessary, to bring it into line with modern developments.

The first thirteen chapters, occupying 205 pages, are devoted to "chemical physics," the subjects dealt with being line spectra and the Bohr theory, X-ray spectra, radioactivity, atomic numbers, isotopes and isobares, positive rays, the segregation of isotopes, the atomic nucleus and the electronic arrangement of the atoms. The section on line spectra and one dealing with cosmic rays are new to this edition. The second half of the book deals, in nine chapters, with topics more closely related to the ordinary work of the chemist, *i.e.* the recently discovered elements hafnium, illinium, rhenium and masurium, active hydrogen, hydrides, the Donnan equilibrium, flame reactions, emission band spectra, continuous emission spectra (the last four sections being new), and Tesla-luminescence spectra.

This book will appeal to the student who wishes to obtain information on these subjects. Prof. Stewart writes in a clear style which makes the reading of his book a pleasure as well as a source of profit; it would, for instance, be difficult to find a more lucid description of the elements of line spectra and the Bohr theory of the atom than that which appears in Chapter II. The book also supplies the demand of the student for information on subjects not usually found in the standard text-books, such as, for example, the preparation and properties of the hydrides.

In the reviewer's opinion, however, some of the material hardly conforms to the title "recent advances"; radioactivity, the radioactive emanations, Moseley's experiment and Aston's mass-spectrograph are subjects which have been assimilated into the body of chemical theory and find their place in the text-books.

On the other hand, the trend of modern quantum mechanics is of sufficient novelty and importance to deserve mention. The reviewer was disappointed to note the absence of any reference to recent developments in the theory of solutions; the name of Debye does not appear even once in the index. A chapter dealing with surface films and adsorption would also have been welcomed.

Apart from this, however, Prof. Stewart is to be congratulated on maintaining his high standard. The book is, with few exceptions, free from typographical errors, and the indexes are adequate.

R. A. ROBINSON.

NON-INTERPOLATING LOGARITHMS, COLOGARITHMS AND ANTILOGARITHMS. By FREDERICK W. JOHNSON, M.A. San Francisco: Simplified Series Publishing Co. 1930. Price \$2.25.

The method of interpolation used in the usual form of logarithm table has been dispensed with in this book. The author has made use of the seven-place logarithms and antilogarithms of numbers, and by rounding them off has obtained tables accurate to the 5th and 4th figures, respectively. His method of arrangement permits one to read the figure required directly, no additions being necessary in order to arrive at the completed figure. The results obtained are for this reason more accurate than those given by the usual tables.

The marginal thumb index provided, together with the system of arrangement, ensure a saving of time that is further accentuated by the introduction of the table of cologarithms (logarithms of the reciprocals of numbers). The use of cologarithms makes it possible to solve fractions involving a number of factors in the numerator and denominator by the one operation of addition.

The average chemist, whose work rarely requires an accuracy involving more than 4 significant figures, will find the usual 4-figure mathematical tables more convenient for his purpose. The physicist, statistician and others using 5-figure logarithms with sufficient frequency, will find this work of value.

A table of International Atomic Weights is given, with the logarithm and cologarithm of each. Tables of certain of the more frequently used chemical, physical and mathematical constants are provided, together with a useful table of metric conversion factors; in all cases the logarithm and cologarithm are given.

In addition, use is made of the functions, S, T, etc., which represent

$$\log \frac{\sin x}{x \text{ (in seconds of arc)}}, \log \frac{\tan x}{x},$$

etc., respectively, for accuracy in the determination of the trigonometrical functions of small angles and angles approaching 90°.

There is a well-written, lucid introduction explaining the use of the various tables.

M. BOGOD.

# THE ANALYST

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## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

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### Standards for Jams.

THE Council of the Society has had under consideration the action that should be taken by Public Analysts with regard to jams which bear the name of a fruit or fruits, but do not bear the descriptive labels agreed upon with the Food Manufacturers' Federation (*ANALYST*, 1930, 55, 694), *i.e.* jams which bear neither the "full fruit" nor the "lower fruit" standard label.

The fruit content of "full fruit" jam has been accepted by the Council as the minimum amount of fruit which should be present in an article sold, without qualification, as "jam," the word "jam" being used in conjunction with any named fruit. It follows that any named fruit jam containing a lower proportion of fruit than this should, for the information of the purchaser, be suitably labelled. The "lower fruit standard" declaration provides for such a jam, and provides also for the requisite information being given to the purchaser.

The Council is of opinion that any jam which

- (1) contains less of the named fruit or fruits than the proportions specified for that jam in the full fruit standard specification, and
- (2) is sold without a declaration disclosing its lower fruit content,

should, for the purposes of the Food and Drugs (Adulteration) Act, 1928, be deemed to be deficient in fruit.

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## The Determination of the Hydroxyl Content of Organic Compounds: Estimation of Castor Oil.

By S. MARKS, M.Sc., A.I.C., AND R. S. MORRELL, M.A., Ph.D., F.I.C.

(Read at the Meeting, February 4, 1931.)

THE object of this investigation was to discover a reliable method for the determination of the hydroxyl ( $-OH$ ) content of several complex compounds of oily consistence which had been isolated in the course of the oxidation of  $\beta$ -elaeostearin from tung oil (Morrell and Marks, *J. Oil and Colour Chem. Assoc.*, 1927, **10**, 197; 1929, **12**, 183; *J. Soc. Chem. Ind.*, 1931, **50**, 29; cf. ANALYST, 1929, **54**, 503). The usual process of acetylation followed by hydrolysis with potassium hydroxide, or a modification of this process, could not be employed, because (1) the compounds under investigation were decomposed by alkali, and (2) their behaviour, on being heated, was uncertain, and it was, therefore, necessary to conduct the estimation at the lowest possible temperature; if possible, at room temperatures.

The methods recommended by André (*Chem. Umschau*, 1925, **32**, 177) and by Verley and Bölsing (*Ber.*, 1901, **34**, 3354) were first examined, using castor oil, which consists mainly of the glyceride of the hydroxy acid, ricinoleic acid, and which should give approximately 5.2 per cent. of  $-OH$  content.\* The results given by both these methods at various temperatures were low and discordant. Investigation of the modification of Bölsing's process put forward by Peterson and West (*J. Biol. Chem.*, 1927, **74**, 379) was satisfactory. This consists in treating the hydroxyl compound under examination with a mixture of excess of acetic anhydride and pyridine; the excess of acetic anhydride is titrated with alkali. The following compounds were examined by this process to test its suitability: (i) Castor oil, dried over exsiccated sodium sulphate; (ii)  $\beta$ -naphthol (A.R.); (iii) vanillin, recrystallised (m.pt.  $80^{\circ}C.$ ); (iv) guaiacol, redistilled (b.pt.  $202^{\circ}C.$ ).

About 2 grms. of a mixture consisting of 1 part of acetic anhydride and 3 parts of pyridine were added to 0.5 to 1 gm. of the compound in a pyrex flask. After standing for the periods and at the temperatures set out in the table below, the reaction product was washed into a beaker with ice-cold water and titrated rapidly with  $N/2$  potassium hydroxide solution and phenolphthalein.

\* Pure triricinolein  $(C_{19}H_{35}O_2)_3C_3H_5$  requires 5.47 per cent. of  $-OH$ .

The table shows some of the results obtained.

### DETERMINATION OF HYDROXYL CONTENT.

Substance.	Temperature. °C.	Duration.	Result. Per Cent.	Calculated.
(1) Castor oil .. .. .	room	24 hours	5.1	about 5.2
	37	24 "	5.1	
	100	15 minutes	5.0	
(2) $\beta$ -Naphthol .. .. .	room	48 hours	11.7	11.8
	37	24 "	11.8	
	100	15 minutes	11.6	
(3) Guaiacol .. .. .	room	24 hours	10.4	13.7
	"	120 "	13.5	
	37	24 "	11.3	
	100	15 minutes	13.3	
(4) Vanillin .. .. .	room	24 hours	10.8	11.2
	"	120 "	11.2	
	37	24 "	10.6	
	100	15 minutes	11.2	
(5) Methyl ester of oxidised $\beta$ -elaeostearic acid, $C_{18}H_{38}O_6(CH_3)_2$ ..	37	120 hours	4.6	4.6
	37	48 "	4.0	
	room	48 "	4.7	
	followed by			
	50	18 "		
(6) Ditto, after hydrogenation, $C_{18}H_{30}O_6(CH_3)_2$ .. .. .	37	120 "	4.3	4.7
	room	120 "	4.0	

Examination of the figures shows that: (a) Castor oil and  $\beta$ -naphthol are comparatively readily acetylated, and ordinary room temperatures can be employed. Castor oil was, therefore, used as a control in the examination of the new oily compounds of unknown constitution, referred to above. The period of exposure was 120 hours, as this apparently sufficed for all the substances examined.

(b) Castor oil,  $\beta$ -naphthol, guaiacol and vanillin give satisfactory results by immersion in boiling water for 15 minutes (Verley and Bölsing, *loc. cit.*). This process, therefore, becomes available for the rapid determination of the purity of these substances, and of others which are not decomposed when heated. The method is particularly valuable for castor oil, which is distinguished from other fixed oils by its remarkably high hydroxyl content. The method is much more rapid and simpler than the determination of the acetyl value by the ordinary procedure.



## The Investigation of Japanese Beeswax.

By HARUICHI IKUTA.

(Read at the Meeting, March 4, 1931.)

SINCE the report of Brodie (*Annalen*, 1848, 67, 180) there have been published about 140 papers dealing with the properties and constituents of beeswax, but, with few exceptions, these were mainly concerned with European beeswax, and hitherto there have been only two reports on Japanese beeswax, one by Shibasaki (*ANALYST*, 1915, 40, 549) and the other by Ueno (*ANALYST*, 1915, 40, 346). According to the investigations previously made, the differences in general properties between Oriental and European beeswaxes were considered to be due to the species of bee, but this had not been established. Ueno confirmed this theory by the results of his experiments on Corean and Ogasawara beeswaxes (*J. Chem. Ind., Tokyo*, 1918, 21, 319). At the present day, the species and distribution of bees are as follows:

No.	Species of Bee.	Geographical distribution.
1	<i>Apis dorsata</i> , Fabricius, 1793	} India, Java, Malayan Peninsula, Borneo.
2	<i>Apis florea</i> , Fabricius, 1787	
3	<i>Apis indica</i> , Fabricius, 1798	East India, China, Japan, South Sea.
4	<i>Apis mellifica</i> , L., 1761	Europe, Africa, America.

The oriental beeswax (both Indian and Chinese), which is known by the name of "Ghedda Wax," is secreted by the first three species mentioned above (Nos. 1, 2 and 3). The genuine Japanese beeswax is secreted by the Japanese honey-bee (*Apis indica*, Fab., var. *Japonica*, "Rads.," 1887), which occurs only in Japan and Corea, and closely resembles the oriental beeswax in its chemical and physical characteristics. During the last fifty-five years, however, European beeswax, produced by bees of species No. 4, which had been imported from Hawaii and America in 1876, 1879 and 1899, has also become a Japanese product, and recently this species has been widely and increasingly distributed throughout Japan. On the other hand, in consequence of certain difficulties in culture and of the poor yield of honey, the Japanese honey-bees have lately very much decreased; it is even said they will soon cease to exist, owing to the competition of the European bees.

For this reason, I have made an investigation of the characteristics and composition of the original Japanese beeswax, while there is still the opportunity.

**NATURE OF SAMPLES.**—The following table gives the districts in Japan where the samples were collected, the appearance of the sample as received, its floral origin, and the appearance of the purified wax derived from it. The wax, separated from the comb by melting with boiling water and straining through calico, was boiled several times with water to remove all soluble impurities, and was finally separated, filtered and dried at 100° C.

TABLE I.

Sample No.	District from which sample was collected.	Appearance of sample as received.	Appearance of purified wax.	Species of bee.	Artificial comb.	Floral origin.
1	Matsuyama	Clear yellow	Almost white	Doubtful	None	Doubtful
2	Iyo	Black honeycomb	Clear orange	Japanese	„	Orange, haze tree
3	Wakayama	Brown	Pale brown	Doubtful	Present	Obscure
4	Itami	Yellow	Almost white	Italian	„	Rape, clover, orange
5	Ogasawara	Yellow	Pale yellow	„	None	Wild plant
6	Sado	Yellow honeycomb	Yellow	„	Present	Rape, plum, camellia
7	Osaka	Dark brown	Brown	Cross-breed	„	Doubtful
8	Miyazaki	Yellow	Pale yellow	Doubtful	Doubtful	„

No cross-breed between the Japanese and the European honey-bee can be produced, and, therefore, a cross-breed of honey-bee in Japan must be obtained with *Apis mellifica*, L. (mainly Italian and Carniolan).

**GENERAL PROPERTIES.**—In the following table, the acid and saponification values were determined by Bohrish and Kürschner's modified process (*Pharm. Zentralbl.*, 1910, [25], 20), and the acetyl value was derived from Cook's formula (*J. Amer. Chem. Soc.*, 1922, 44, 392):

$$\text{A.V.} = \frac{V' - V}{1 - \lambda V_{10}^{-3}}$$

where V is the saponification value of original wax, V' the saponification value of the acetylated wax, and  $\lambda$  the ratio; that is,

$$\frac{\text{C}_2\text{H}_5\text{O}}{\text{KOH}} = 0.7488.$$

The determination of the unsaponifiable matter was effected by taking the mean values found by the method of Donath (Grün, *Analyse der Fette und Wachse*, Vol. I, 207) and of Leys (*Chem. Zentralbl.*, 1912, ii, 456).

TABLE II.

Sample No.	Sp. gr. ( $d_4^{100}$ ).	Melting point. °C.	$n_D^{70}$ , calculated to $n_D^{40}$ .	Acid value.	Ester value.	Saponification value.	Ratio No.	Iodine value (Wijs).	Acetyl value.	Un-saponifiable matter. Per Cent.
1	0.8168	64.5-65.0	1.4557	7.5	75.6	83.1	10.1	11.3	20.7	58.3
2	0.8232	65.0-65.5	1.4560	5.4	79.2	84.6	14.5	14.0	18.7	56.5
3	0.8148	62.0-62.5	1.4552	16.4	83.4	99.8	5.1	8.2	12.2	50.5
4	0.8152	62.5-63.0	1.4554	18.6	74.5	93.1	4.0	9.9	12.4	48.6
5	0.8141	62.5-63.5	1.4554	19.8	74.0	93.8	3.7	8.5	13.1	49.5
6	0.8132	62.0-62.6	1.4546	19.4	74.1	93.5	3.8	7.2	14.4	48.6
7	0.8108	62.5-63.0	1.4545	15.5	73.0	88.5	4.7	9.7	10.9	51.2
8	0.7980	61.5-62.5	1.4535	9.7	52.7	62.4	5.4	7.6	11.4	68.2

For comparison with these figures, constants of oriental beeswax, as recorded by different observers, are given in the following table:

Wax.	Sp. Gr.	Melting point.	Acid value.	Ester value.	Saponification value.	Ratio No.	Iodine value.	Authority.
<i>Apis florea</i>								
max.		68	8.9	123.8			11.4	
min.	—	63	6.1	80.8			6.0	
mean	—	64.2	7.5	95.6			8.0	
<i>Apis dorsata</i>								
max.	—	67	10.2	97.8			9.9	} Hooper, <i>Agric. Ledger</i> , 1904 (7).
min.	—	60	4.4	69.5			4.8	
mean	—	63.1	7.0	89.4			6.7	
<i>Apis indica</i>								
max.	—	64	8.8	95.9			9.2	
min.	—	62	5.0	84.0			5.3	
mean	—	63.2	6.8	89.6			7.4	
East Indian								
max.	—	63.5	8.9	99.5	106.1	14.9	9.3	} Berg: Lewkowsch, II, p. 924.
min.	—	62.5	6.3	86.2	93.6	10.0	7.1	
mean	—	63.0	7.0-7.5	89-94	96-101.5	12.5-13.5	8.5-8.7	
Indian	( $d_4^{15}$ )							
mean	0.9652	61.4	5.8	92.1	97.9	16.7		} Roberts and Islip (ANALYST, 1922, 47, 246).
Japanese	( $d_4^{100}$ )							
max.	0.8207	66.5	8.19	95.14	103.34	14.6	14.14	
min.	0.8135	64.9	5.61	80.45	86.35	11.19	10.18	} S. Shibasaki (ANALYST, 1915, 40, 549).
mean	0.8160	65.9	6.40	83.44	89.85	13.14	12.27	
Corean	( $d_4^{100}$ )							
max.	0.8358	66.0	7.7	81.34	87.72	17.63	12.93	} S. Ueno (ANALYST, 1915, 40, 343).
min.	0.8090	65.0	4.46	74.24	79.24	10.23	10.04	
mean	0.8229	65.6	5.85	78.71	84.56	13.45	11.41	

The following remarks may be made regarding the constants of the Japanese beeswax:

Sample No. 1. The employment of artificial comb in the collection of this

sample is doubtful, but, in view of the general properties, this wax would have been considered to be genuine Japanese beeswax.

No. 2. In obtaining this wax no artificial comb was employed, and the constants for this sample agree approximately with those previously attributed to genuine Japanese beeswax.

Nos. 5 and 6. These samples were secreted by European bees (Italian honey-bee), and, therefore, they have the normal constants of European beeswax.

Nos. 3, 4 and 7. These samples, as they have a somewhat high ratio number, are not pure European beeswax, like Nos. 5 and 6.

No. 8. Taking into consideration all the results, such as low specific gravity, melting point, saponification value and refractive index, and high percentage of unsaponifiable matter, the conclusion must be drawn that this sample of wax is adulterated with a large quantity of hydrocarbon.

ARTIFICIAL COMB.—Seventy-three years have passed since the invention of artificial comb by J. Mehring, in 1857, and, at the present day, having regard to the economic and cultural points of view, no one can afford to dispense with its use.

In the earliest years of the invention, artificial combs were prepared from a mixture of beeswax and beeswax substitutes. At first, ceresin was used for this purpose, but, at present, paraffin wax or stearic acid has taken its place, and such a product must, of course, be looked upon as adulterated. Therefore, it is important, in an investigation of beeswax, that the wax taken as sample should be tested for impurities at the outset, otherwise the outcome will be an inaccurate report. For instance, I examined an artificial comb in the case of sample No. 4 (Table II), and its analytical values were as follows:

Sp. gr. ( $d_4^{100}$ )	..	..	0.8193	Iodine value (Wijs)	..	7.2
M.pt. ( $^{\circ}\text{C.}$ )	..	..	64–64.5	Unsaponif. matter (per cent.)	..	55.6
Ref. index ( $n_D^{40}$ )	..	..	1.4552	M.pt. of unsap. matter	..	75–76 $^{\circ}\text{C.}$
Acid value	..	..	10.7	M.pt. of sap. matter	..	54–55 $^{\circ}\text{C.}$
Ester value	..	..	88.9			
Saponif. value	..	..	99.6			
Ratio number	..	..	8.3			

From the above results it will be seen that the artificial comb of No. 4 consisted of pure beeswax, or of a mixture of beeswax and some other wax. According to my investigation, the wax of No. 4 contained about 10 per cent. of artificial comb, and, therefore, if we calculate the properties of beeswax No. 4, which contains 10 per cent. of artificial comb, the acid, ester and saponification values will be, respectively, 19.4, 73.0 and 92.4, and the ratio number 3.8. These constants agree approximately with the constants of genuine European beeswax No. 5, which did not contain artificial comb.

PROPERTIES OF SAPONIFIABLE SUBSTANCES.—I saponified 20 grms. of the sample with 50 c.c. of *N*-alcoholic potash and 20 c.c. of benzene in a special separation flask. When the saponification was completed, hot water was added, and the boiling was continued under a reflux condenser for a few minutes. The soap solution was drawn off while hot, and was treated with benzene three times, and the saponifiable substances were then separated in the usual manner by boiling with acid. These saponifiable substances were considered to be adulterated with large quantities of fatty acids and very small quantities of resinous matters and unsaponifiable matters. The constants of these saponifiable substances are shown in the following table:

TABLE III.

Sample No.	Melting point °C.	Neutralisation value.	Iodine value.	Mean molec. weight.
1	54 to 55	187.2	9.1	299.2
2	49.5 to 50.5	188.5	10.1	297.6
3	57 to 58	184.5	7.8	304.1
4	58 to 59	182.2	8.7	307.9
5	57 to 57.5	176.5	7.2	317.8
6	57.5 to 58	180.1	7.2	311.5
7	56.5 to 57	172.1	9.6	326.0
8	58 to 59	170.5	7.5	329.0

PROPERTIES OF UNSAPONIFIABLE MATTER.—The unsaponifiable substances separated from saponifiable matter were white or pale yellow solid masses. Their characteristics were as follows:

TABLE IV.

Sample No.	Melting point °C.	Saponification value.	Iodine value.	Acetyl value.
1	73.5 to 74.5	3.8	10.6	91.0
2	74.5 to 75.4	3.5	17.1	93.8
3	72.5 to 73.1	3.3	9.3	91.5
4	72.5 to 73	4.5	8.4	90.0
5	74 to 75	3.1	8.4	90.4
6	74 to 74.6	3.2	6.8	90.5
7	72 to 72.5	2.5	10.7	82.5
8	69 to 69.6	1.8	9.1	50.6

PROPERTIES OF WAX-ALCOHOL AND HYDROCARBONS.—To separate the unsaponifiable hydrocarbons in beeswax from the alcohols, I used the method of Leys (*Chem. Zentralb.*, 1912, ii, 456), as adopted in the technical standard analysis of oils and fats in Germany (*Chem. Umschau*, 1929, 207). The principle of this method is based on the insolubility of the hydrocarbon in a mixture of fuming hydrochloric acid and amyl alcohol, in which wax-alcohol is soluble. This method of separation is not chemically exact, but, as it is simple, and yields the wax-alcohol without any change, it is convenient in practice.

The results are given in the following tables:

TABLE V.

Sample No.	Melting point of alcohol. °C.	Wax-alcohol		Saponi- fication value.
		Acetate.		
		Melting point. °C.		
1	76.5 to 77	62.5 to 63		112.4
2	77 to 77.6	62.5 to 63.2		114.3
3	74.5 to 75.5	69 to 61		110.8
4	75 to 75.5	61 to 61.5		110.1
5	75.5 to 76	63 to 63.5		113.6
6	75 to 75.5	62 to 63		108.1
7	75 to 76	61 to 62		109.5
8	74 to 75	61.5 to 62		119.8

TABLE VI.

Sample No.	Melting point. °C.	Hydrocarbons.	
		Iodine value (Wijs).	
1	65 to 66	17.4	
2	64.5 to 65	26.7	
3	64 to 65	14.8	
4	63.5 to 64.5	16.3	
5	62.5 to 63	17.2	
6	63 to 64	15.4	
7	63 to 63.5	14.2	
8	64 to 64.5	9.1	

The value given by Lewkowitsch (*Oils, Fats and Waxes*, I, 617) for the acetyl value of the beeswax alcohols, namely, 99 to 103, seems to be erroneous, as the theoretical value for myricyl alcohol is 116.7, and that of ceryl alcohol is 132.3. According to investigations hitherto made (*J. prakt. Chem.*, 1912, 86, 184), beeswax contains myricyl and ceryl alcohols, but East Indian wax contains only ceryl alcohol. The melting point and iodine value of the hydrocarbons are given respectively as 49.5 to 59.2° C., and 20 to 22 by Lewkowitsch (*loc. cit.*). For these reasons, it will be clearly seen that the wax-alcohol and hydrocarbons of the foregoing tables were still not perfectly separated. However, further investigations on this problem are in progress.

SUMMARY.—The results obtained appear to indicate that:

1. The differences in general properties between European and Japanese beeswaxes depend on the species of the bees rather than on the floral origin of the honey.

2. In this research, the samples No. 1 and No. 2 were the genuine Japanese beeswaxes which were secreted by the Japanese bee (*Apis indica*, Fab., var. *Japonica*, "Rads," 1887), and the other six samples were produced by the European bees (*Apis mellifica*, L., 1761) and their cross-breeds.

3. It is important that the influence of the use of artificial comb should be borne in mind.

I wish to express my sincere thanks to Professor Seiichi Ueno for his kind guidance throughout this work, and also to Mr. Suekichi Suwa for the supply of the materials used in this research.

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## A Method for the Determination of Small Quantities of Hydrogen Sulphide: the Determination of Sulphur in Small Samples of Steel.\*

By S. G. CLARKE, Ph.D., A.I.C.

It occasionally happens that analyses of steels are required when the amount of sample available is too small for the ordinary processes of steel analysis to be of service. In certain cases, such as the study of segregation in steel, metallurgists are handicapped by lack of suitable methods for the analysis of small samples. This is more especially true in the determination of sulphur and phosphorus because of the very small percentages of these elements which are in question. The ordinary methods of determination call, at any rate in the case of sulphur, for a sample weight of 5 grms. of steel. For the purposes for which the method to be outlined was developed, the sample weight available for a determination was 0.1 gm.

There are two main methods for the determination of sulphur in steel: the gravimetric barium sulphate process, and the evolution process, which consists in dissolving the steel in a non-oxidising acid and determining the liberated hydrogen sulphide by a volumetric method, such as titration with iodine solution. The latter type of method is not so generally applicable to all kinds of steel as the former, but apart from certain alloy steels, *e.g.* those containing tungsten, molybdenum or copper, the evolution process is generally regarded as giving results which are quite as satisfactory as those from the gravimetric process, and it is, moreover, more rapid.

In view of the urgency of finding a method suitable for these small samples of steel it was decided to explore the possibilities of the evolution process, as being the most likely direction in which a workable process might be found. A process was worked out on the following lines:

\* Communication from the Research Department, Woolwich.

**OUTLINE OF THE PROCESS FOR STEEL.**—By employing a special apparatus of simple design it is possible to dissolve the small sample of steel out of contact with the air in an atmosphere of hydrogen. It has been found that by using dilute sulphuric acid (1:3), which possesses advantages over other acids in the process to be described, the whole of the sulphur in the sample is evolved, and can be collected as sulphide in a small volume of dilute sodium hydroxide solution; it can then be readily determined by a new colorimetric method which depends on measuring the amount of iodine (in carbon tetrachloride solution) used in oxidising the sulphide in slightly acid solution.

**COLORIMETRIC METHODS FOR DETERMINING HYDROGEN SULPHIDE.**—In order, when working with a decigram sample of steel, to obtain results which would be of an order of accuracy comparable with that expected of the usual processes, the method employed would have to be reliable to within  $\pm 3$  thousandths of a milligram of sulphur on a total weight of under one-tenth of a milligram. An investigation of colorimetric processes available has revealed that they are valueless for the present purpose.

The conversion of dimethyl-*p*-phenylenediamine into a blue dye by hydrogen sulphide and ferric chloride has been found to possess the drawback that the colours produced are not strictly reproducible, nor are they due entirely to the reaction product of hydrogen sulphide; the reaction is, moreover, not nearly sensitive enough.

An experimental survey of metal sulphides as a basis for a colorimetric process for determining traces of hydrogen sulphide showed, at first, promising results as regards sensitiveness in the case of antimony and, in particular, lead. These methods, however, are quite empirical, since the depth and quality of the colour produced from a certain amount of hydrogen sulphide seem to be entirely dependent upon arbitrary conditions prevailing in the reaction solution. Attention has already been drawn (Evers, *ANALYST*, 1920, **45**, 391) to the influence of the *pH* value of the solution upon the intensity of the colour of the lead sulphide when used to determine lead; in the present case it has been found that, in alkaline solution, a colour is produced which is much darker than in dilute acetic acid solution. Attempts were made to obtain consistent results, working with a standardised solution of sodium sulphide (prepared in the manner indicated below) by controlling what variables could be controlled, *e.g.* the concentration of salts, acetic acid, the lead chloride added, the gum arabic protective colloid used, the temperature of the solution, etc., and by adhering to a rigid routine in the order of mixing reagents. Even in these circumstances the results were barely within the limits of accuracy desired, the colorimetric comparisons being carried out with the aid of a Klett colorimeter. No success could, however, be achieved in the application of this lead sulphide method to the determination of the sulphide disengaged from steel by acid and absorbed in alkali, largely owing to the practical difficulties in producing the colour under precisely the conditions prevailing in the preparation of a standard solution.



**THE NEW COLORIMETRIC METHOD.**—It is known that iodine can react quantitatively with hydrogen sulphide in aqueous solution, with the formation of sulphur and hydriodic acid, and this is the basis of a volumetric process commonly used for determining sulphide. If, however, the iodine consumed could be determined colorimetrically it might be expected that the reaction could be used for determining smaller amounts of sulphide than would be feasible in the volumetric method. A process on this basis would have the advantage of being an absolute one if the amount of sulphide could be calculated from the amount of iodine used.

It has been found that minute amounts of sulphide can be determined by shaking a slightly acid (sulphuric) solution containing the hydrogen sulphide in a closed flask with a known excess of iodine in carbon tetrachloride, and determining colorimetrically the residual amount of iodine by comparison with another known amount of iodine in carbon tetrachloride. Before applying this method to steel analysis it was tested upon a standardised sulphide solution. The following method was adopted:

A standardised sodium sulphide solution (approx.  $N/200$ ) was run into a small flask of about 50 c.c. capacity, having a well-fitting glass stopper, 4 drops of dilute sulphuric acid (1:3) were added, standardised iodine in carbon tetrachloride (approx.  $M/200$ ) was immediately run in, and the flask stoppered. It was shaken vigorously for about 20 seconds, an amount of carbon tetrachloride was added from a burette to bring the total volume of this liquid in the flask up to 10 c.c., and the contents of the flask were poured into one cup of a Klett colorimeter. Into the other cup was run a suitable volume of the standardised iodine solution, together with the amount of carbon tetrachloride, to bring the volume to 10 c.c. The ratio of the depths of the two solutions which transmitted the same amount of light was then found, the unused iodine being then readily calculated. Some results which were obtained are recorded in Table I.

The results show that the amount of iodine consumed for a certain weight of sulphide is independent of the initial excess of iodine used over the range examined. It may, therefore, be concluded that the same reaction occurs between iodine and hydrogen sulphide when these are shaken together in a mixture of carbon tetrachloride and water as when the organic liquid is absent, and that the amount of sulphide present can be calculated from the amount of iodine used according to the ordinary equation.

The sulphide solution used was prepared by passing about 50 c.c. of hydrogen sulphide into 250 c.c. of cold, freshly boiled water, to which a little sodium hydroxide had been added. The solution was standardised by adding a distinct excess of iodine (0.01  $N$ ) to a portion immediately after it had been acidified, allowing the mixture to stand for five minutes, and determining the excess of iodine with standardised very dilute thiosulphate solution, with starch as indicator. The solution of iodine in carbon tetrachloride was made and standardised as described later for use in the process for steel.

TABLE I.

Added.				Found.	
Sulphide solution. c.c.	Sulphide sulphur. Mgram.	Iodine solution. c.c.	Iodine solution remaining. *c.c.	Iodine solution consumed. c.c.	Sulphur. Mgram.
1.0 of 0.0048 <i>N</i>	0.077	2.0	$\frac{30}{27.0} \times 1.0 = 1.11$	0.89 of 0.0053 <i>N</i>	0.076
1.0 <i>..</i>	0.077	3.0	$\frac{15}{21.3} \times 3.0 = 2.11$	0.89 <i>..</i>	0.076
1.0 <i>..</i>	0.077	5.0	$\frac{30}{22.0} \times 3.0 = 4.08$	0.92 <i>..</i>	0.078
0.5 <i>..</i>	0.039	1.0	$\frac{30}{28.3} \times 0.5 = 0.53$	0.47 <i>..</i>	0.040
0.5 of 0.00635 <i>N</i>	0.051	1.0	$\frac{25}{35.0} \times 0.5 = 0.36$	0.64 of 0.00533 <i>N</i>	0.055
0.5 <i>..</i>	0.051	2.0	$\frac{30}{21.5} \times 1.0 = 1.40$	0.60 <i>..</i>	0.051
0.5 <i>..</i>	0.051	3.0	$\frac{20}{16.9} \times 2.0 = 2.37$	0.63 <i>..</i>	0.054
1.0 <i>..</i>	0.102	3.0	$\frac{20}{22.3} \times 2.0 = 1.80$	1.20 <i>..</i>	0.102
1.0 <i>..</i>	0.102	5.0	$\frac{30}{15.8} \times 2.0 = 3.80$	1.20 <i>..</i>	0.102

\* This column shows the ratios of the depths (in mm.) of the solutions used for comparison and the experimental solutions, when the field viewed in the colorimeter was uniform, multiplied by the number of c.c. of standard iodine solution contained in the 10 c.c. of comparison solution, giving the number of c.c. of standard iodine solution remaining after interaction with the sulphide.

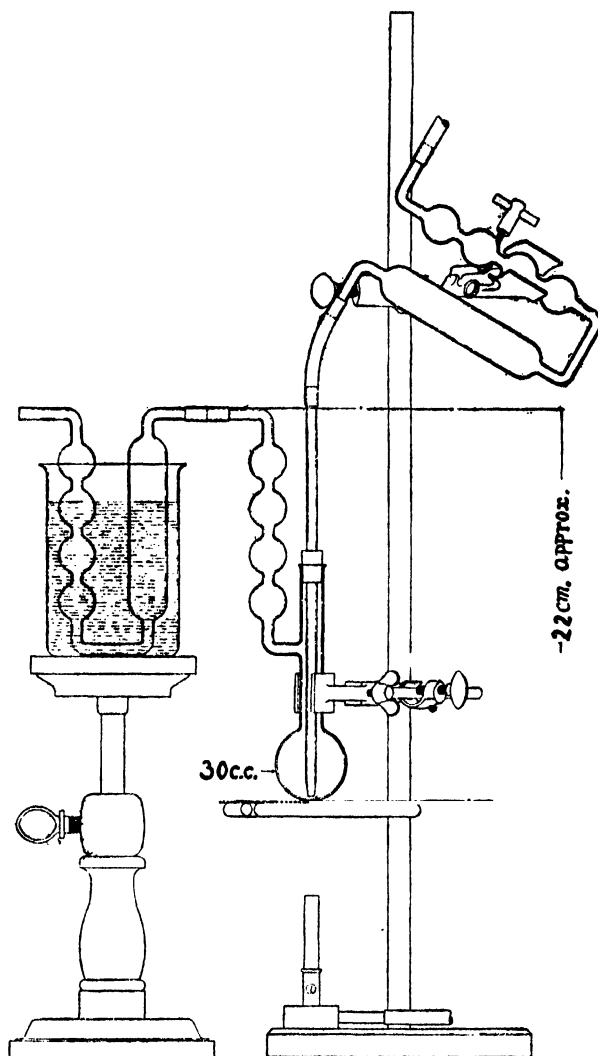
† Rather brownish colour of the experimental solution.

**THE EVOLUTION OF HYDROGEN SULPHIDE FROM SMALL STEEL SAMPLES.**—Preliminary experiments showed that it was not possible to obtain quantitative recovery of sulphur by treatment of the steel with acid unless this was done in an atmosphere of hydrogen. In order to avoid wastage of hydrogen, definite volumes were used from an approximately graduated aspirator; for the initial removal of air from the apparatus, 500 c.c. of hydrogen was found to be a safe amount, and a similar quantity was used for sweeping out after the steel had dissolved. It may be noted that the hydrogen used contained a fraction of one per cent. of oxygen, but, in view of the satisfactory results obtained, it would appear that this concentration of oxygen is not harmful. Before entering the apparatus the gas was passed through lead acetate solution and then through water.

The apparatus, which had to be designed for this work, and the construction of which is shown in the diagram, comprises the following parts:

(a) *The Evolution Flask.*—This is a flask of 30 c.c. capacity. It has a vertical side arm, carrying four bulbs, which has a two-fold function: (i) As a reflux condenser, preventing appreciable loss of water during the solution of the

steel; (ii) as a very efficient preventer of acid spray, charged with ferrous salt, being carried into the receiver. This apparatus can be readily made by cutting off the side tube from a Wurtz flask close to the join, and fusing on, in its place, the limb carrying the bulbs of a Mitscherlich absorption U-tube which has been



severed at the base. This flask is closed by a cork carrying a glass tube which reaches nearly to the bottom of the flask, and the lower end of which is somewhat constricted.

(b) *The Acid Container* is a Mitscherlich absorption tube in which the acid is placed at the outset. It is connected with the entry tube of the above flask by

about three inches of black rubber tubing, so that, after air has been removed from the apparatus by the passage of hydrogen, acid can be run on the steel merely by releasing the clamp and tilting the container.

(c) *The Absorption Tube* is another Mitscherlich absorption tube in which the small volume of dilute sodium hydroxide is placed; it is connected by black rubber tubing with the side arm of the evolution flask. The tube stands in a beaker of cold water supported as shown in the diagram.

(d) *The Bunsen Burner*.—This is a burner of the small type, such as is used for heating the mirror-tube in electrolytic arsenic determinations.

A number of different acids were tried, but the only one found suitable was dilute (1:3 by volume) sulphuric acid. Hydrochloric acid, even when more dilute than the constant-boiling mixture, gave rise to trouble, owing to irregular amounts of it passing over, and partly or wholly neutralising the necessarily small quantity of alkali in the absorption tube. Hydrobromic acid (1.49 sp. gr.) was found to be more satisfactory than hydrochloric acid by reason of its higher boiling point, but, nevertheless, a little found its way into the alkali; here it was found to interfere with the subsequent iodine colorimetric method for sulphide, owing to its causing a very marked reduction in the partition coefficient of iodine between carbon tetrachloride and the aqueous solution, and thereby causing loss of iodine.

**METHOD FOR THE DETERMINATION OF SULPHUR IN STEEL.**—The following method was adopted for the determination of sulphur in small samples of steel:

The sample of steel (0.1 grm.), in the form of fine drillings, is brushed into the flask, care being taken to prevent any particles sticking to the neck of the flask. Dilute sodium hydroxide solution (2 c.c. of a 10 per cent. solution) is run from a pipette into the Mitscherlich absorption tube, the addition being made to the limb carrying the bulbs. Dilute sulphuric acid (5 c.c., 1:3) is similarly run into the upper Mitscherlich tube, which acts as the acid container. The whole apparatus is now assembled, and the acid container adjusted to such an angle to the vertical that gas can pass freely through it without passing through the volume of acid which it contains.

Hydrogen is passed in a rapid stream through the apparatus for 1 to 2 minutes, approximately 500 c.c. being used. The rate of supply of hydrogen is then reduced to a flow of about 2 to 3 bubbles per second. The acid container is removed from its clamp and tilted so that the acid runs into the flask, and immediately it has done so the container is replaced in the clamp and the burner lighted under the flask, the liquid in which is rapidly heated to boiling. The flame is then reduced to maintain only a steady boiling, when, with fine drillings of medium carbon steels, complete solution of the sample (with the exception of a little carbonaceous matter) will take place in less than two minutes.

When the steel has dissolved, the rate of flow of hydrogen through the apparatus is again increased to that used in the initial sweeping out, and about the

same quantity allowed to pass.\* The flame is then extinguished, the hydrogen supply stopped, the rubber tube joining the acid container to the leading tube to the flask is disconnected, to prevent sucking back, and the absorption tube is detached.

The liquid in the absorption tube is poured (from the limb which was attached to the flask) into a small flask which has a *well-fitting* glass stopper (a 50 c.c. volumetric flask was used); about an equal volume of water is used for rinsing out the absorption tube, the rinsings being added to the main volume in the flask.

One c.c. of dilute sulphuric acid (1:3) is added, and the flask is placed in cold water and moved about gently therein for about half a minute in order to cool after the neutralisation of the alkali. Without delay exactly 2 c.c.† of the standard solution of iodine in carbon tetrachloride is run into the flask from a small accurate 10 c.c. burette, and the stopper immediately inserted.

The flask is now shaken vigorously for about 30 seconds. Exactly 8 c.c. of carbon tetrachloride (or 9 c.c. if only 1 c.c. of the standard solution had been used) is then run into the flask; the contents are mixed and poured into the cup of a Klett colorimeter, no rinsing of the flask being necessary.

COLORIMETRIC COMPARISON.—One c.c. of the standard iodine solution is run into the other colorimetric cup, 9 c.c. of carbon tetrachloride are added, and the solution mixed (0.5 c.c. and 9.5 c.c. of iodine solution and carbon tetrachloride, respectively, are taken when only 1 c.c. of iodine was used in the above method). The colorimeter having previously been adjusted as regards zero points and equality of lighting of the tubes, the depth of the sample solution required to balance any particular depth of the above comparison solution is measured.

The iodine remaining (as c.c. of standard solution), after reaction with the hydrogen sulphide, is simply

$$\frac{x}{y} \times n$$

where  $x$  = depth (in mm.) of the comparison solution,

$y$  = depth (in mm.) of the sample solution,

$n$  = c.c. of standard iodine solution (generally 1) added to the comparison tube.

\* During the experimental work a second absorption tube, containing an alkaline lead solution, was connected with the main alkali absorption tube shown in the diagram. Absorption of the hydrogen sulphide by the alkali in the first tube was thereby shown to be complete, in spite of the very rapid flow of hydrogen used in the sweeping out, because the same alkaline lead absorption tube was used each time and no darkening of the solution could be detected at the time of completion of the work.

† Or 1 c.c. when the sulphur content of the steel is known definitely to be low, say, less than 0.03 per cent. This gives a little greater accuracy in the colorimetric determination with steels low in sulphur, but 2 c.c. would serve quite well for all steels up to 0.08 per cent.

The value of  $y$  taken should be the mean of several readings, and the ratio  $\frac{x}{y}$  should agree closely with a fresh ratio obtained by using a different value for  $x$ .

The amount of standard iodine solution used in the reaction with the hydrogen sulphide is obtained by deducting the above value from the amount originally used, whence the amount of sulphur can be readily calculated from the strength of the iodine solution employed:

$$1 \text{ c.c. of } N/200 \text{ iodine} = 0.080 \text{ mgrm. of sulphur.}$$

**THE STANDARD IODINE SOLUTION.**—This is prepared by dissolving 0.32 gm. of iodine in carbon tetrachloride; this can be carried out rapidly by covering the iodine with carbon tetrachloride, warming on a steam bath until an opaque solution is obtained, and then treating the remaining iodine with fresh portions of the solvent until all is dissolved. The cooled solution is diluted to 500 c.c. It is standardised by titration with arsenious acid ( $N/100$ ) as follows:—Twenty c.c. of the iodine solution are run into a 500 c.c. flask, and 100 c.c. of water and 20 c.c. of dilute (5 per cent.) potassium iodide are added, followed by a few grms. of sodium bicarbonate. About 9 c.c. of the arsenic solution are added, the solution is well shaken, and the titration finished, after the addition of starch solution, up to the practical disappearance of the blue colour; very vigorous shaking is necessary near the end-point. The addition of potassium iodide is necessary to reduce the partition coefficient of iodine between carbon tetrachloride and water.

**IMPORTANT POINTS IN THE PROCESS.**—(a) Steels which are low in carbon, and contain a relatively small percentage of impurities, generally dissolve much more slowly in acid than ordinary steels. This is the case, *e.g.* with British Chemical Standard “A<sub>2</sub>” steel (S, 0.020; C, 0.037), and results for sulphur first obtained with such steel by this method were too high; this was traced to the fact that the acid had become concentrated by evaporation during the dissolving process, and the presumption was that traces of a reducing gas, other than hydrogen sulphide, thereby became liberated; a crystalline precipitate (ferrous sulphate) was noticed in the flask in the experiments in which high results were obtained. Good results were obtained with such steels when the flame under the flask was turned very low during the dissolving of the steel, so as to avoid undue concentration of the acid. The formation of crystals in the flask, before it cools down after an experiment, is to be regarded as an indication of danger.

(b) The standard comparison solution in a colorimeter cup will alter appreciably in iodine concentration if kept for, say, more than 15 minutes, because carbon tetrachloride is a somewhat volatile liquid. Incorrect results will then be obtained in the determination of the iodine remaining after interaction with hydrogen sulphide, since this solution does not lose carbon tetrachloride at the same rate as the comparison solution, owing to its being covered with a layer of water.

Table II contains results obtained in the process with British Chemical Standard steels.

TABLE II.

Steel.	Weight taken. Grm.	Iodine solution.* c.c.	Iodine solution† remaining after reaction. c.c.	Iodine solution consumed. c.c.	Sulphur found. Per Cent.	Mean certificate result.	
						Gravi- metric.	Volu- metric.
Blank—no steel		1.0	$\frac{30.0}{30.0} \times 1.0 = 1.0$	nil			
A <sub>1</sub>	0.1	1.0	$\frac{30.0}{38.0} \times 1.0 = 0.79$	0.21	0.019	0.020	0.021
„	0.2	2.0	$\frac{30.0}{19.0} \times 1.0 = 1.58$	0.42	0.018		
O <sub>1</sub>	0.1	1.0	$\frac{25.5}{20.0} \times 0.5 = 0.64$	0.36	0.031	0.032	0.032
	0.2	2.0	$\frac{30.0}{23.0} \times 1.0 = 1.30$	0.70	0.030		
H <sub>1</sub>	0.1	2.0	$\frac{30.0}{19.5} \times 1.0 = 1.54$	0.46	0.039	0.041	0.042
	0.1	1.0	$\frac{30.0}{29.5} \times 0.5 = 0.51$	0.49	0.042		
	0.2	2.0	$\frac{30.0}{30.4} \times 1.0 = 0.99$	1.01	0.043		
N <sub>1</sub>	0.2	3.0	$\frac{20.0}{22.0} \times 2.0 = 1.82$	1.18	0.050	0.050	0.051
V‡	0.1	2.0	$\frac{30.0}{23.2} \times 1.0 = 1.29$	0.71	0.060	0.063	0.062
P	0.1	2.0	$\frac{30.0}{26.3} \times 1.0 = 1.14$	0.86	0.073	0.073	0.073

\* 0.00530 N; 1 c.c. = 0.085 mgrm. of sulphur.

† This column has the same significance as in Table I.

‡ Chromium-vanadium steel: V., 0.27 per cent.; Cr, 0.86 per cent.

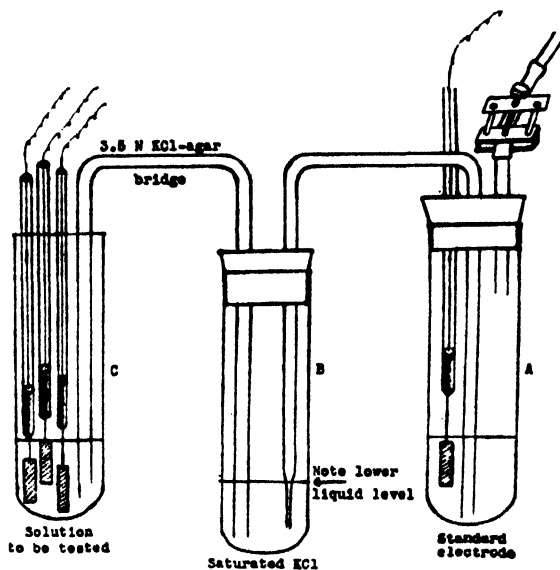
The results obtained by working with different quantities of sample (0.1 grm. and 0.2 grm.) show satisfactory agreement.

## The Use of the Quinhydrone Electrode.

By GEORGE M. MOIR, PH.D., F.I.C.

(*Pedler Research Scholar of the Institute of Chemistry.*)

THE determination of the  $pH$  value, by means of the hydrogen electrode, is a slow process owing to the fact that the gas must be passed through the liquid for about 5 to 10 minutes before a reading can be taken, and then the electrode must be cleaned and boiled before being ready for another determination. The quinhydrone electrode introduced by Büllmann permits determinations of  $pH$  on the acid side to be carried out very much more rapidly. For many purposes it is



distinctly more accurate than colorimetric methods, and is scarcely less rapid. The reliability of any method of determining  $pH$  is, however, governed by the nature of the solution being investigated, and cases are known in which even the hydrogen method must give place to the colorimetric procedure. The quinhydrone electrode has the special advantage that it can be used according to the method of Knudsen (*Z. Unters. Lebensm.*, 1925, 50, 300), for solid or semi-solid material, such as cheese or gelatin, etc.

Instead of the usual type of standard electrode vessel to which is fused a side-tube, I have devised a simple arrangement consisting of an ordinary test-tube of stout glass, about 1"  $\times$  4", fitted with a rubber stopper in which three holes are bored (A, Fig. 1). Through these holes pass, respectively, the glass tube of the



electrode, an inverted U-tube, the outer end of which ends in a capillary, and a third short tube with rubber tube and screw clip attached; if necessary, the apparatus can be made air-tight by applying a trace of vaseline to the cork. This apparatus has three advantages: first, it is inexpensive, and any breakages can be easily and rapidly replaced; second, it is flexible, for the U-tube can be rotated to any required position; third, it can be readily slipped into a metal rack in a water-bath or thermostat. The diagram shows how connection is made with the solution to be tested, which is usually placed in another short test-tube (C) standing in the metal rack. The intermediate tube (B), which contains saturated potassium chloride solution, is usually put outside the water-bath. It is fitted with a cork having large holes, or slots cut in the side, so that the tubes can be easily slipped in and out.

The actual electrode in A may be of bright platinum, since it is always in contact with the same solution, and is not so liable to "poisoning"; but if difficulties are encountered, a gold one may be found better. The electrodes for use in the solution to be tested are preferably made of gold foil (about  $1.0 \times 0.5 \times 0.025$  cm.), to which gold wire (S.W. Gauge 21) is attached, first by hammering, and then by heating just to redness. By the same method the gold wire is then attached to a platinum wire to be fused into a glass tube. Some workers use gold-plated platinum electrodes on account of the risk of melting the gold during flaming, but this gold plating requires renewing from time to time. All-gold electrodes can be flamed without difficulty, provided this is done at a distance from the bright light of a window, so that care can be taken not to heat beyond the first sign of redness. The flaming must be done with an alcohol lamp, to avoid the risk of "poisoning" by coal-gas impurities.

The frequent flaming may cause cracks to develop in the tube into which the platinum wire is fused. Care should be taken to obtain suitable glass tubing for this purpose, in order to avoid loss of time due to frequent cracking; in fact, the process of fusing the platinum properly into tubes—simple as it appears—may very well be delegated to an expert. Abnormal potentials are sometimes caused by leakage of liquid through cracks, which I have found to be most readily detected by keeping the electrodes, when not in use, in a test-tube with strong nitric acid. This gradually passes through the crack and attacks the mercury within, producing a white salt. The mercury used to put in these tubes ought to be clean, pure, and dry, and is best kept in a bottle fitted with dropping-tube and rubber teat, so that small quantities can be transferred without loss.

If by accident an electrode becomes amalgamated, the mercury can be removed by flaming, but on occasion blackish deposits remain—probably due to copper which may have reached the mercury from a connecting wire. These deposits can be removed by plunging the electrode while almost red-hot into strong nitric acid. Scrubbing with a clean brush and soap and water has been recommended for removing sticky material from the electrodes prior to flaming. Another method is to place the electrode in a tube with nitric or chromic acid

set in a boiling water-bath, and sometimes it may be necessary to use all these methods repeatedly. In other cases, failure to give correct results is due to the mercury in the tube becoming moist (especially with acid fumes), whereby a small mercury-copper cell results and causes an abnormal potential.

Connection between B and C is made by means of an agar bridge, which is prepared by adding 5 grms. of agar-agar to 100 ml. of 3.5 *N* potassium chloride solution, and heating in a large test-tube (or beaker) immersed in boiling water. Several U-tubes of suitable size are prepared, clean and dry. To one end of each is fitted a piece of rubber tubing and clip, so that it can be filled by suction, and the clip applied when full. The other end must be left in the test-tube until the whole mass is quite solid, when it may be removed, and, after detaching the rubber tube, it should be kept until required with both ends in test-tubes of saturated potassium chloride solution. Before using, the end which is to go in the solution to be tested should be left for ten minutes in distilled water and rinsed clean. This is of importance, because very small quantities of potassium chloride are able to cause considerable reductions in the *pH* value. The advantage of an agar bridge is that it minimises the risk of potassium chloride reaching the solution to be tested. In fact, it is possible that some of the slight differences which Lester (*J. Agric. Sci.*, 14, 634) found between the quinhydrone and the hydrogen electrode may have been due to this cause. Billmann (*Trans. 2nd Comm. Intern. Soc. Soil. Sci.*, 1927, B, 236) has drawn attention to the risk of siphoning occurring, and for this reason the liquid level in B should be distinctly lower than that in either of the other two tubes.

The difficulties associated with the preparation of a standard calomel electrode for reference purposes can be avoided by the use of a solution containing potassium chloride (0.09 *N*) and hydrochloric acid (0.01 *N*) in the presence of undissolved quinhydrone. Although Veibel (*J. Chem. Soc.*, 1923, 123, 2203) found that the potential of this mixture remained constant for several days, it is preferable to renew it daily. The vessel A should be rinsed once or twice with small quantities of this solution, which are blown out through the U-tube. It is then filled about half full, and a small amount of quinhydrone added (0.1 grm. is usually sufficient). After replacing the cork, the U-tube is filled by blowing gently, and the clip at once closed. Air bubbles above the capillary greatly reduce the sensitiveness of the apparatus. If the cork fits well, little or no leakage should take place from the capillary. After using the apparatus, A should be disconnected from B if no further measurements are to be made for a few hours. Immediately after disconnecting, the clip on A should be opened slightly and a few drops allowed to run out of the capillary, so that any strong potassium chloride solution which may have diffused in will not be allowed to flow back into the apparatus. This precaution is essential if the reference electrode is to be maintained correct for more than one day.

I have found one electrode in A sufficient, but Lester's plan of having three in the solution to be tested was adopted. Thus, when one became "poisoned" it

was at once observed because it gave a different reading from the others. "Poisoning" did not often occur when dealing with clear solutions, but liquids like milk, especially after the protein had been coagulated, gave more trouble. In such cases, washing the electrodes, first under the tap, and then with distilled water, did not suffice, but, in addition, flaming was often necessary after each determination. In order to obtain good results in solutions or mixtures containing clotted material, it may be necessary to use a larger vessel for C and to stir the liquid mechanically, as Lester did.

Every day before measuring an unknown  $pH$  a solution of known  $pH$  should always be tested. For this purpose, 0.05 *N* potassium phthalate, which has a  $pH$  of 3.97, is convenient. "Standard acetate" mixture, which is 0.1 *N* with respect to both acetic acid and sodium acetate, and which has a  $pH$  of 4.626, is easily made up from standard acid and sodium hydroxide solutions of about half-normal strength. A few ml. of the solution to be investigated are placed in C (which need not be quite dry except for highly accurate measurements), the agar bridge inserted, and about 0.1 grm. of quinhydrone added with a spatula. The electrodes are at once inserted and used to stir the mixture briskly, and the potentiometer reading taken as soon as possible (because of the fact that a drift takes place after a time in certain solutions). When the value obtained for a solution of known  $pH$  differs by more than a millivolt from what is expected, an investigation and cleaning of the apparatus and solution should be carried out. The formula for calculating the  $pH$  when using the reference electrode described above is:

$$pH = 2.03 + \frac{E}{0.0577 + 0.0002(t - 18)}$$

where  $E$  is the voltage (in volts) and  $t^\circ C.$  is the temperature. By drawing a graph of  $pH$  against E.M.F. at  $18^\circ C.$  the  $pH$  values can be read off at a glance. Lines can be drawn to obtain the values at adjacent temperatures.

The statement is generally made that the availability of the quinhydrone electrode is confined to the acid side, but Büllmann himself has compared it with the hydrogen electrode, and obtained results agreeing well, even at  $pH$  values above 7.0. Lava and Hizon (*Chem. Abst.*, 1929, 23, 538) found it satisfactory up to  $pH$  9.0, provided that the potentiometer readings were taken promptly after adding the quinhydrone. I have not come across records of trials with it at higher  $pH$  values.

Many workers have published results which they have obtained by means of the quinhydrone electrode (*cf.* Watson, *Ind. Eng. Chem.*, 1927, 19, 1272), but I have found the papers cited in the references especially useful. A number of important details given above have been taken from Büllmann's paper, which may not be so readily available as others.

## A Simple, Inexpensive Quinhydrone Cell for Rapid Work.

By J. G. DAVIS.

MOIR has described a modified quinhydrone electrode (preceding paper). The apparatus now described is a further simplification, and was prepared for determining the  $pH$  of a large number of samples of liquid or solid substances without the aid of salt-agar bridges or glass taps.

The half cell consists of a pyrex tube, about  $\frac{1}{8}$  inch in diameter, which has been drawn out into a fine jet and bent over as shown in Fig. 1. It is important that

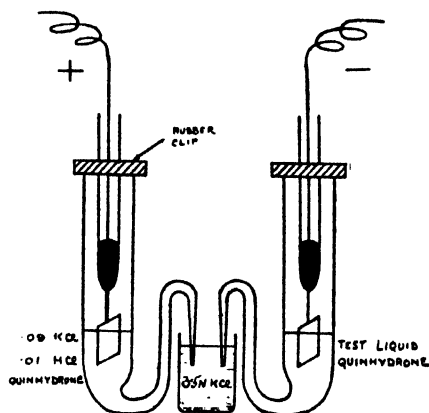


Fig 1

the jet be about  $\frac{3}{4}$  to 1 inch above the bottom of the tube. To prepare the standard quinhydrone half cell, pour some hydrochloric acid potassium chloride reagent (9 parts of 0.1  $N$  KCl and 1 part of 0.1  $N$  HCl, very accurately standardised) into the cell, add about 0.05 gm. of quinhydrone, shake, allow to settle, and then gently blow over. No bubbles should be allowed in any part of the cell. The amount of liquid put in should be so gauged that when blown over, the level is about  $\frac{1}{8}$  inch above the jet, thus preventing siphonage. For  $pH$  determinations of liquids a similar cell is prepared, except that the liquid under test is used instead of the hydrochloric acid-potassium chloride solution.

Gold electrodes, after having been rinsed in distilled water and flamed to a dull red heat (momentarily) in a pure alcohol flame, are immersed in the cells, the jets of which are dipped into 3.5  $N$  potassium chloride solution just before a reading is taken and removed immediately afterwards.

Sensitivity is good, and a constant value should be obtained in 2 to 3 minutes from the time of mixing-in the quinhydrone. For solids, either of two methods may be used:

(1) The solid is ground in a mortar with quinhydrone, pressed into a short piece of tubing (a one-inch section of a test tube  $\frac{1}{8}$  inch in diameter), and the electrode inserted in this "mush," which is then allowed to dip into saturated KCl and contact made as before (Fig. II.).

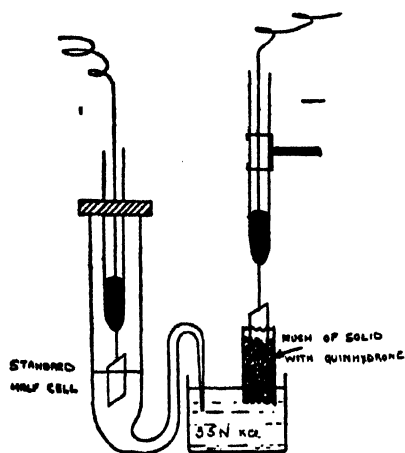


Fig II

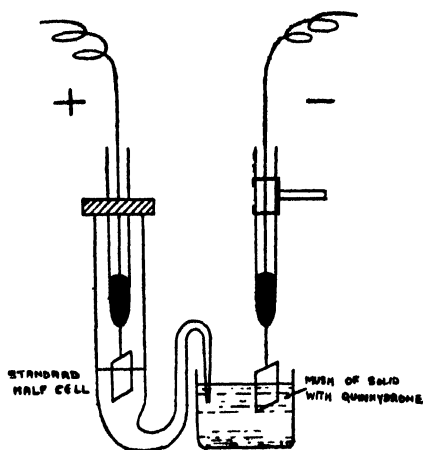


Fig III

(2) The jet of the standard half cell is allowed to touch the "mush" in a crucible about 1 inch from the electrode. No appreciable error was detected when using well-buffered solids over a range of pH from 3 to 7 (Fig. III).

The cells may be emptied by jerking the liquid out and then blowing back *through* the jet.

The half cells can be obtained from Mr. W. J. Nelson, 77, Streatham Vale, S.W.16; price 1s. 6d. each.

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING.  
THE UNIVERSITY, READING.

## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### OSMIUM TETROXIDE POISONING.

I HAVE been unable to find any record of fatal cases due to exposure to the fumes of osmium tetroxide, but indirectly one case of fatal poisoning has come to my notice. This case was referred to by Mr. C. M. Hoke in an article appearing in the *Jewelers' Circular*. My interest lies particularly in determining the pathology both of the acute condition and of chronic poisoning, with the expectation of subsequently developing some method of neutralising the effects.

It may not be generally known that inhaling the fumes of osmium tetroxide in high concentration is a very dangerous procedure; and, if the effect on human beings is at all analogous to that on experimental animals, it would seem probable that some cases of fatal poisoning must have occurred in the past. In animals, at least, the cause of death is a pneumonia which appears very shortly after their exposure to the fumes.

Any information that can be given me in this connection will be deeply appreciated.

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### THE POSSIBLE EFFECT OF SULPHUR DIOXIDE WHEN USED AS A PRESERVATIVE FOR DRIED FRUITS, ETC.

IN the course of routine work on products preserved with sulphur dioxide, and more particularly with fruit juices, the presence of hydrogen sulphide has made itself evident to the users by its odour and the flavour of the juices after dilution.

Under some conditions the presence of hydrogen sulphide was suspected in dried fruits also, and it was in connection with the examination of some hundreds of such samples that the following information was gathered.

For the prevention of the growth of moulds and the elimination of living organisms, it is necessary to "sulphur" such fruits as raisins, sultanas, apricots, peaches, apples and pears before they are marketed, and, for the preservation of fruit juices, either sulphur dioxide or benzoic acid may be employed.

In the course of work undertaken in Spain, France, Italy and Greece, I have been able to reduce the proportions of sulphur dioxide to a minimum without diminishing the market value or keeping qualities of the dried fruits, but the moist pulps need different treatment, and the existing Regulations permit the use of larger proportions. It is in these products, which are used for the preparations of syrups and "soft" drinks, that the effect of sulphur dioxide is most pronounced.

In order to demonstrate the possible state or situation of the sulphur dioxide in dried fruits, the following examination was made of a shipment of sultanas:

Sulphur dioxide before washing	..	375	parts per million.
" " after washing	..	320	" " "
" " in the washings	..	45	" " "

This seems to indicate plainly that most of the gas is present as an actual compound of the aldoses with the sulphite.

It might be argued that such a compound was entirely harmless to consumers of such fruit, but such a contention would not hold good if the dried fruit were used for making, say, artificial wines, mince meat or sauces, and the same disability is attached to the use of fruit pulps containing sulphite, either added purposely or left in barrels accidentally after cleaning, for I have found that a yeast fermentation becomes possible in diluted solutions; and, when that is active, the sulphur compounds are reduced to hydrogen sulphide.

To prove that this was possible, I expressed the juice from some Almeria grapes, sterilised it by boiling, and divided it up in flasks. To one, sulphur dioxide was added in the form of sodium bisulphite to approximately the proportion of 750 parts per million, as permitted in the Regulations. To another nothing was added. Both were then inoculated with a platinum wire dipped in an active growth of yeast. Other flasks were used as controls, and the neck of each was covered with a piece of lead paper and an inverted beaker.

After 24 hours at 65° F. an active fermentation was set up in the plain juice, but nothing had commenced in those containing sulphur dioxide. These were then diluted with more sterilised juice and left for several days under the same conditions, the results being as follows:

Flasks, to which no sulphur dioxide had been added, produced no coloration whatever on the lead paper, but with those containing the diminished proportion of sulphur dioxide, active fermentation had started, and the lead papers were marked with a perfectly defined black disc on an unstained white background.

Some difficulty was experienced in obtaining dried fruit free from sulphur dioxide for similar tests, but such samples as I have tested and found free from it yield no blackening whatever with lead paper when they are suitably mixed with water and fermented.

A fermentation test for the presence of sulphur dioxide, is too complicated for routine work, but I found that "sulphured" fruit and "sulphited" pulps have the disability that, when moistened or diluted with syrup, they develop an objectionable amount of hydrogen sulphide if once fermentation by yeast is allowed to commence.

It is necessary, therefore, to prevent the possibility of conditions which will permit fermentation, and, among other precautions, such products should be examined for the presence of active yeast cells; and if such are found present, the syrups or mixtures should be pasteurised before bottling.

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## THE DETERMINATION OF PHOSPHORUS IN STEEL, ALLOY STEELS AND CAST IRON.

MR. ETHERIDGE, in his paper on "The Determination of Phosphorus in Steel, etc." (ANALYST, 1931, 14), begins by remarking that "Phosphorus is oxidised to ortho-phosphoric acid in nitric acid solution," and on page 16 he remarks that "oxidation

with permanganate is necessary to destroy organic matter. . . . The omission of permanganate leads to low results, possibly due to the interference of organic substances. . . ."

My own experience has led me to confirm the experience of others, that phosphorus is not completely oxidised to orthophosphoric acid when steel or iron is dissolved in *dilute* nitric acid, and that the addition of potassium permanganate is made for the main purpose of converting the phosphorus into orthophosphoric acid; the oxidation of carbonaceous matter is of decidedly secondary importance. Carbonaceous matter may be oxidised by means of several other oxidising agents, but very few of them will convert the phosphorus into orthophosphoric acid.

If a mild steel with very little carbon, but, say, 0.1 per cent. of phosphorus, is dissolved in dilute nitric acid, it will be found that, although there is little or no carbonaceous matter present, the precipitation of the phosphorus will be seriously low without the addition of permanganate, thus illustrating my point that the function of the permanganate is primarily to convert the phosphorus into orthophosphate. It may be mentioned, however, that if a steel is dissolved in dilute nitric acid or *aqua regia*, the complete conversion of the phosphorus into orthophosphoric acid will take place if the solution is evaporated and the residue baked.

Another interesting fact is that, so far back as 1885, J. Mackintosh showed that phosphorus in a solution of steel in hydrochloric acid may be oxidised to orthophosphoric acid by means of a stream of sulphur dioxide gas without oxidising the ferrous iron.

On page 16, Mr. Etheridge remarks that "it would be possible to add ammonium nitrate at this stage, but this would require less nitric acid to be used in dissolving the steel, which would be undesirable for rapid solution and oxidation of the carbides formed." He may be interested to know that in the "Analoid" method for phosphorus in steel, 2 grms. of drillings are dissolved in no more than 30 c.c. of nitric acid (sp. gr. 1.20), that solution takes place very quickly, and there is no difficulty with regard to oxidation of the carbides formed. Thousands of tests which have been made by works' chemists by this method over a period of ten years or so, support this statement.

On page 18, the author refers to my paper on the effect of titanium on the determination of phosphorus, but goes on to say that "It is fortunate that it is not usual to encounter low-phosphorus iron with much more than 0.01 per cent. of titanium." If, however, he refers to p. 111 of *The Proceedings of The Cleveland Institution of Engineers*, 1919-20, he will see that I have analysed 16 definite brands of pig iron from different parts of Great Britain, and that the titanium in these ranged from 0.08 to 0.42 per cent., the average being about 0.15 per cent. One feature of my paper was to show that most haematite pig irons contain a moderate amount of titanium, which calls for attention in the determination of phosphorus.

On p. 20, the author refers to vanadium and tungsten steels, and states that in tool steels with 15 per cent. of tungsten some of the tungsten is precipitated, and that this always carries down some phosphorus. It may be mentioned, however, that in a process which I have worked out, good results are obtained by dissolving the steel in nitric acid, followed by a liberal supply of potassium permanganate. This method, which has been in use for some ten years, was given in detail in the *J. Iron & Steel Inst.* (1926, No. 1, p. 464).

The reference to Messrs. Rooney & Clarke's paper, given on page 21 of *THE ANALYST*, contains a misprint—the year should be 1926, not 1925.



Taken as a whole, however, Mr. Etheridge's paper contains much useful information, and these remarks are not intended in any way to detract from its value.

N. D. RIDSDALE.

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WITH reference to Mr. Ridsdale's remarks on the use of potassium permanganate, while there is no doubt that low results are obtained if it is not used, the mechanism of its action is not clear. Most authorities consider that meta-phosphoric acid is formed, and that potassium permanganate assists in transforming this into ortho-phosphoric acid. In both acids the phosphorus is fully oxidised, and the transformation of meta-acid into ortho-acid is a hydration effect, not oxidation, hence the difficulty of explaining why permanganate is necessary. According to the text-books meta-phosphoric acid is quickly transformed into ortho-phosphoric acid by boiling in the presence of mineral acids, but this is retarded by organic acids. It is possible, therefore, that the permanganate, by destroying these acids formed from the carbon of the steel, may thus indirectly assist in the transformation of meta- to ortho-acid.

Dr. B. S. Evans has recently carried out some experiments which appear to show that phosphorous acid is formed on dissolving steel in nitric acid, and he has further found that dilute nitric acid has practically no oxidising action on phosphorous acid. Bauer and Deiss (*Sampling and Analysis of Iron and Steel*) also state that phosphorous acid is formed. Brearley and Ibbotson (*Analysis of Steel Works' Materials*) state that phosphorus in steel is not completely oxidised by nitric acid alone. It seems probable that phosphorous acid is formed in the first place and partly oxidised to phosphoric acid by oxides of nitrogen produced by dissolving the steel in nitric acid, but it is necessary to complete the conversion to phosphoric acid with an energetic oxidiser such as potassium permanganate. Dr. Evans has also shown that chromic acid is not a sufficiently powerful oxidiser in this case, having little or no action on phosphorous acid.

As regards the use of a smaller amount of nitric acid than is specified in the paper, while 30 c.c. would be satisfactory in many cases, there are alloy steels which deposit carbides which are difficult to dissipate, and for these an excess of nitric acid is an advantage.

My experience with haematite cast iron is, no doubt, considerably less than Mr. Ridsdale's, but I have not encountered any with more than traces of titanium.

With regard to Mr. Ridsdale's method for high tungsten steels, in which tungsten is held in solution by using less nitric acid throughout, it is admitted that tungstic acid is liable to separate, to some extent, in the later stages, and phosphorus must be recovered from this precipitate, as was pointed out by Rooney and Clarke (*J. Iron and Steel Inst.*, No. I, p. 466). Furthermore, the lower acidity favours co-precipitation of arsenic, which may demand a reprecipitation. Also, it is by no means safe to use the same values for the volumetric solutions unless they are standardised under the same conditions, as the precipitate of ammonium phosphomolybdate does not appear to be quite in accordance with the formula when precipitated from solutions containing only a small excess of nitric acid.

I wish to thank Mr. Ridsdale for his interesting comments.

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## Notes from the Reports of Public Analysts.

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### COUNTY OF LANCASTER.

#### ANNUAL REPORT OF THE COUNTY ANALYST FOR THE YEAR 1930.

OF the 5303 samples examined, 4987 were taken under the Food and Drugs Act, and of these, 128 were adulterated. In the County of Lancaster it is now the general practice to take formal samples of milk and informal samples of other articles. If the informal sample is suspicious or adulterated a formal sample is taken from the same source.

**FREEZING-POINT TEST OF MILK.**—Most of the milks received during the year which were deficient in solids-not-fat were submitted to this test in cases where it was possible to obtain an "appeal-to-cow" sample. The corresponding results in 32 cases (given in tabular form) show that the information obtained by the freezing-point test was identical with that gained from comparison with the corresponding "appeal-to-cow" sample.

**DAY-TO-DAY VARIATION IN THE MILK OF TWO HERDS.**—A daily examination was made of the milk from a large herd and from a small herd at the Lancashire County Council Farm at Hutton, near Preston. The larger herd, which was sampled from July 5th, 1926, to November 15th, 1926, consisted of 39–45 cows, all non-pedigree shorthorns. The smaller herd, which also consisted of non-pedigree shorthorns, was sampled from September 24th to November 14th, 1928. It consisted of 11–14 cows.

The total solids were determined by weighing, often in duplicate. The fat was determined by duplicate Leffmann and Beam determinations in all cases; four determinations were carried out in all, if the first two did not agree. The refraction readings were obtained by the copper sulphate serum method (ANALYST, 1927, 52, 193) with the Zeiss immersion refractometer at 20° C. The specific gravity was determined, and in each case the calculated solids agreed well with the experimental figure.

Although the series taken from the larger herd extended from the hot weather of July to the cold weather of November, it will be observed that the solids-not-fat were practically constant throughout the whole of this time, whilst the variations in fat were unimportant. The results are given in full in tables in the Report.

**FACED PEARL BARLEY.**—An informal sample of pearl barley was found to be faced with maize starch to the extent of 1.3 per cent. A subsequent formal sample was similarly faced to the extent of 1.5 per cent., and the manufacturers were cautioned. Another sample faced to the extent of 0.2 per cent. with maize starch was also returned as adulterated.

**CHALK IN GROUND GINGER.**—Of the 98 samples examined, 2 were returned as adulterated. One of these contained 7.86 per cent. of total ash, 4.92 per cent. of ash insoluble in water, and 0.28 per cent. of sand. The insoluble ash consisted

largely of chalk, which is said to be added to prevent the growth of weevils. Most of the ground ginger of commerce is free from added chalk, and, therefore, it would appear that this addition is not necessary. It may be alleged that some types of ginger are more prone to the attacks of weevils than others; but in this case it would appear desirable to inform the purchaser of the particular type of ginger supplied, and the necessity for such treatment, as some purchasers might prefer to avoid weevils by purchasing the type immune from their attack.

JAM.—During the five years ended 1930, the number of samples of jam examined was 243, of which 12 were returned as adulterated. Eight contained foreign fruit, three contained glucose syrup when the label claimed that the jam was prepared from fresh fruit and pure sugar, and one contained excessive sulphur dioxide.

Many of the samples have been examined for their optical rotation, both before and after inversion, in 10 per cent. aqueous solution in a 200 mm. tube. The direct reading with mercury green light has varied between  $+6.55^\circ$  and  $+0.11^\circ$ , and the invert reading between  $-0.30^\circ$  and  $-3.56^\circ$ . The percentage of total sugars, as given by the refractometer, has varied between 58 per cent. and 74 per cent. In certain cases the amount of insoluble solids has been determined. The following are some of the results which have been obtained with single fruit jams:

*Insoluble Solids in Jams. Per cent.*

	Number.	Average.	Highest.	Lowest.
Blackcurrant ..	10	2.15	2.98	1.29
Strawberry ..	20	1.31	2.28	0.59
Raspberry ..	11	1.85	2.60	0.72
Apricot ..	4	1.25	2.76	0.68
Damson* ..	13	0.88	1.73	0.49

\* Without stones.

POLISHED RICE.—Since the year 1926, 195 samples of rice have been examined in the County Laboratory, of which 16 have been coated with varying amounts of talc. Of these 16 samples, only three have contained more than 0.5 per cent. Some years ago it was by no means unusual for rice to be polished with talc, but in more recent years, as shown by the figures above, this practice has almost entirely ceased. This proves that it is unnecessary. The suggested limit of 0.5 per cent. made to the Local Government Board is most certainly too high; in fact there are many reasons why the use of talc should be entirely prohibited. It has been alleged that the facing of rice and other cereals is intended to prevent the attacks of weevils. It is very doubtful whether facing is of any value to prevent such attacks, and the fact that most of the rice now sold is free from such facing shows that the bulk of manufacturers find it to be unnecessary. The uncoated samples contained from 0.19 to 0.42 per cent. of ash.

G. D. ELSDON.

## General Medical Council.

### PHARMACOPOEIA COMMISSION.

#### REPORT OF THE COD-LIVER OIL COLOUR TEST SUB-COMMITTEE.\*

SEVERAL modifications of the details of the antimony trichloride test, first proposed by Carr and Price, have been proposed from time to time, but no attempt has been made to show that even by adherence to any one form of the test, the same results are yielded in the hands of different workers. The Sub-Committee set out to determine this and to decide the conditions best calculated to give uniform results. It was found at the outset that when different members of the Sub-Committee examined the same samples of cod-liver oil, their results differed very greatly. The work of the Sub-Committee has, therefore, been directed to discovering the cause of the differences, and in the end they have defined a form of test which if strictly followed will always yield results in near agreement for a given sample of cod-liver oil in the hands of different workers.

In examining cod-liver oil by this test, a blue colour is produced which is compared with graded colour glasses such as the Lovibond colour glasses. The limit recommended in the test as described below corresponds to a colour similar to that of a Lovibond blue glass given the value 6.0.

The test, as recommended by the Sub-Committee, is a limit test, which does not require any precise determination of the blue value of cod-liver oil expressed in terms of a scale like the Lovibond scale. Since, however, this test is often used for making such precise determination, the Sub-Committee wish to point out how desirable it is for different workers to adhere closely to the conditions proposed. They also wish to point out how undesirable is the practice which has arisen of speaking of the colour value of cod-liver oil in terms of blue "units." The Sub-Committee feel that the term "blue unit" should give place to the term "blue value." The figure given to a glass on the Lovibond scale represents merely a grading in a series of glasses, and does not represent an amount of biological activity. The term "unit" used in measuring various therapeutic substances whose activity can only be determined biologically, means a definite amount of biological activity, and its application to the blue glass used for this cod-liver oil test has produced very great confusion of thought.

#### ANTIMONY TRICHLORIDE TEST FOR COD-LIVER OIL.

0.04 grm. of cod-liver oil examined by the following method gives a blue colour not less saturated, that is to say, not paler, than that of a blue glass standardised to have the following properties on the system of colour measurement adopted at the National Physical Laboratory:

Colour quality: 0.137R + 0.271G + 0.592B

Photometric transmission: 34.0 per cent.

In the foregoing specification R, G and B, respectively, denote the colours of monochromatic radiations of wave-length  $0.700\mu$ ,  $0.546\mu$  and  $0.436\mu$ , and the measurements both of colour quality and photometric transmission are presumed to be made with the National Physical Laboratory standard "white" light.

**DESCRIPTION OF TEST.**—Weigh 2.00 grms. into a narrow-necked 10 millilitre measuring flask; fill to the mark with *chloroform* at a temperature of  $20^\circ$  and mix. Measure, at  $20^\circ$ , 0.2 millilitre of the solution so prepared by means not less accurate than a 1 millilitre pipette, the graduated portion of which is at least 15 centimetres long, into a colourless rectangular cell of 10 millimetres internal measurement in the direction of observation (see below). Place the glass cell in a colorimeter designed for matching the colour of the solution against colour glasses. Add rapidly 2.0 millilitres of antimony trichloride reagent, in such a way that the solutions mix. Simultaneously

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\* The Cod-liver Oil Colour Test Sub-Committee was appointed by the Commission to recommend a form of test for cod-liver oil depending on what is known as the antimony trichloride reaction. In appointing the Sub-Committee the Commission wished it to be understood that the inclusion of such a test in the Pharmacopoeia was not to be taken as necessarily indicating that the test afforded a measure of vitamin A content. The test, if included, might prove of value for indicating a limit of deterioration or as indicating a characteristic property of cod-liver oil.

The Sub-Committee consisted of Dr. J. H. Burn (Chairman), with Messrs. Bacharach, Carr, Cocking, Evers, Jowett, Tainsh and Rosenheim, with Dr. C. H. Hampshire (Secretary).

observe the development of a blue colour, which rapidly reaches a maximum and then fades. By means of combinations of graded colour glasses match the colour at the point of maximum intensity. It may be necessary to employ yellow and red as well as blue glasses. In order to obtain an accurate match it may be necessary to diminish the transparency on the side of the cell; this must be done by adding on that side neutral tinted glasses, the value of which should be disregarded.

Several preliminary observations should be made to enable subsequent readings to be taken without undue delay in arranging the glasses, and to determine how long after mixing is the point of maximum intensity of colour. The maximum intensity may develop within ten seconds, but the time varies with different oils. It is of the greatest importance that the observer should satisfy himself that the final match is made at the point of maximum intensity of the blue colour. Neither the reagent nor the solutions should come into contact with rubber.

**ANTIMONY TRICHLORIDE REAGENT.**—A solution of *antimony trichloride* in pure dry chloroform saturated at 20° is prepared in the following way: Wash *chloroform* two or three times with its own volume of distilled water, dry the chloroform over *anhydrous potassium carbonate*; pour off and distil, rejecting the first 10 per cent. of the distillate. During drying and distillation protect the chloroform from light. Wash *antimony trichloride* with the pure dry chloroform until the washings are clear. Prepare a solution, saturated at 20°, of the washed antimony trichloride in the pure dry chloroform. The solution, which must contain not less than 21 and not more than 23 per cent. w/v of  $\text{SbCl}_3$ , should be kept in a well-stoppered bottle of amber-coloured glass.

**Assay.**—Mix 1 millilitre with a solution of 2 grms. of *sodium potassium tartrate* in 20 millilitres of water; rotate the mixture, add 2 grms. of *sodium bicarbonate* and titrate with *N/10 iodine*. Each millilitre of *N/10 iodine* is equivalent to 0.01141 grms. of  $\text{SbCl}_3$ .

#### NOTE BY THE PHARMACOPOEIA COMMISSION.

**COD-LIVER OIL—VITAMIN D.**—The Pharmacopoeia Commission have considered the question of making a requirement for the amount of vitamin *D* in cod-liver oil. The evidence brought before the Commission hitherto has led to the provisional conclusion that it is not necessary to make such a requirement. The main grounds for this conclusion are—(1) That although there may be considerable variation in the amount of vitamin *D* present in different samples, almost all samples contain enough for therapeutic purposes; (2) That the estimation of vitamin *D* is an expensive and time-consuming process which should not be required unless absolutely necessary.

In view, however, of the increasing practice of stating the vitamin *D* potency on the label of bottles of cod-liver oil, and of the desirability of uniformity in the expression of this potency, the Commission are considering an addition to the requirements which will define the Standard Preparation and the Unit, and indicate the methods of assay which are to be used by those who wish to make a biological estimation of vitamin *D* in samples of cod-liver oil. If this addition is made, the Standard Preparation and the Unit will be those recently defined by the Medical Research Council.

## Cyprus.

### ANNUAL REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1929.

THE Government Analyst, Dr. S. G. Willmott, reports that of the 1713 samples examined during the year, 1665 were official, including 406 exhibits in criminal cases and 469 foods and drugs. The number of food and drug samples showed a decrease of 384, compared with the previous year, and the adulteration had increased from 8.3 to 10.5 per cent.

The method of taking official samples of food and drugs in Cyprus leaves much to be desired. First, it is doubtful whether the small sum of £20 is adequate to cover all expenses incurred in the collection of a satisfactory number of samples of food and drugs (including milk and aerated waters) for the whole Island, in any one year.

Secondly, the present practice of the police in sending for analysis large numbers of samples from all six administrative districts at practically the same time, is most unsatisfactory from every point of view. In the past, samples appear to have been submitted only about three times per year.

The result is that, during the intervening period, the public is not protected against the adulteration of their food. This difficulty could be easily overcome, without increase in expenditure, by the simple expedient of taking fewer samples at more frequent intervals throughout the year. For a proper control over the food-supply, some samples should be sent for analysis every week. The present system also has the effect of temporarily dislocating the work of the Laboratory, because anything upwards of 250 samples of food and drugs may accumulate in the course of one week. The examination of such a number involves weeks of work, and could be avoided by the remedy indicated.

Thirdly, the genuineness of the food-supply is well protected by the system of taking surprise samples. The shopkeeper is not likely to practise adulteration if he is aware that an official sample may be taken at *any* time, and not merely at three expected times, as under the present system. This method also allows more latitude of action. For example, it was observed, in the course of a toxicological investigation, that a certain sample of coffee sent in by the police as an exhibit was adulterated with starch. This information was duly communicated to the Local Commandant, Military Police, with the suggestion that an official sample of the coffee in question should be taken under the Food and Drugs Law. The reply received was that no action could be taken in the matter, since the funds allocated for the year were exhausted.

It seems probable that the appointment of a food inspector would eradicate most of the shortcomings of the present system, and without additional expenditure. It should not be difficult to nominate a reliable member of the police force for this special duty, who could be trained to take official samples of the right foods at the right times. There is nothing original in the idea, which has been successfully put into practice in nearly every civilised country, and the Government is urged to give this suggestion the most careful consideration.

**COFFEE.**—Of the 112 samples analysed, 13 were adulterated with roasted wheat ground to a fine powder; this is the usual method of adulterating coffee in Cyprus.

**OLIVE OIL.**—Fifty-one samples were analysed, and the adulteration rose to the unusually high figure of 37·2 per cent., owing to the poor olive harvest of 1928–1929, and the consequent rise in price. The adulterants found were cotton-seed oil, arachis oil and “cocolina.”

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# Fertilisers and Feeding Stuffs.

## Merchandise Marks Act.

STATUTORY RULES AND ORDERS, 1931, No. 171.

### THE MERCHANDISE MARKS (IMPORTED GOODS) No. 7 ORDER, 1931.\*

At the Court at Buckingham Palace, the 20th day of March, 1931.

Present: The King's Most Excellent Majesty in Council.

Whereas by sub-section (1) of Section 2 of the Merchandise Marks Act, 1926 (16 & 17 Geo. 5, c. 53), it is provided that after an enquiry in relation to goods of any class or description has on a reference from the appropriate Department been held by a Committee appointed for the purposes of the said Act and the report of the Committee on the matter has been taken into consideration by the Department, that Department may, unless it appears to them that the trade of the United Kingdom or the trade generally of other parts of His Majesty's Dominions with the United Kingdom would be prejudiced if imported goods of that class or description for use or consumption in the United Kingdom were prohibited to be sold unless they bear an indication of origin, make a representation to His Majesty that it is desirable that an Order should be made under the said Section 2, and His Majesty in Council may thereupon, subject to the provisions of the said Act, make an Order prohibiting the sale or the exposure for sale in the United Kingdom of imported goods of that class or description unless they bear an indication of origin:

And whereas in accordance with the provisions of the said Section an enquiry in relation to the class or description of goods to which the present Order relates, has on reference from the appropriate Department, namely, the Board of Trade (hereinafter called the Board), been held by a Committee appointed for the purposes of the said Act and the report of that Committee has been taken into consideration by the Board:

And whereas by sub-section (5) of Section 2 of the said Act it is provided that if on an enquiry under sub-section (1) of the said Section it appears to a Committee to be desirable that any imported goods should bear an indication of origin at the time of importation, and the Committee so reports to the appropriate Department, that Department, unless, having regard to all the circumstances of the case including the re-export trade of the United Kingdom in that class or description of goods, it considers such action undesirable, may make a representation to His Majesty that it is desirable that the goods should bear an indication of origin at the time of importation, and His Majesty may by Order in Council under the said Section (without prejudice to His Powers under sub-section (1) of the said Section) make provision accordingly:

And whereas it does not appear to the Board that the trade of the United Kingdom or the trade generally of other parts of His Majesty's Dominions with the United Kingdom would be prejudiced if the goods described in this Order imported for use or consumption in the United Kingdom were prohibited to be sold unless they bear an indication of origin, and the Board have accordingly made representations to His Majesty that it is desirable that an Order should be made under the said Section 2.

And whereas the Committee have reported to the Board that it appears to them to be desirable that the goods described in this Order should bear an indication of origin at the time of importation:

And whereas the Board having had regard to all the circumstances of the case including the re-export trade in those goods have made representations to His Majesty that it is desirable that the goods described in this Order should bear an indication of origin at the time of importation:

Now, therefore, His Majesty, by and with the advice of His Privy Council, in pursuance of the powers vested in Him by the said Section and of all other powers enabling Him in that behalf is pleased to order, and it is hereby ordered, as follows:—

1. It shall not be lawful to import into the United Kingdom any of the fertilisers or feeding stuffs specified herein or to sell or expose for sale in the United Kingdom any such fertilisers or feeding stuffs which have been imported unless they bear an indication of origin.

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\* H.M. Stationery Office, Adastral House, Kingsway, London, W.C.2. Price 1d. net.

2. This Order applies to fertilisers and feeding stuffs of the following descriptions:—

- (a) Bone meal and bone flour whether raw degreased or degelatinised;
- (b) hoof meal, horn meal and mixtures thereof;
- (c) meat meal, meat and bone meal and carcase meal;
- (d) dried blood, whether ground or unground.

3. The indication of origin shall be printed, stamped, stencilled, painted or branded on the bag, sack, cask, keg or other container in which the goods are imported or sold or exposed for sale.

4. Nothing in this Order shall require any such fertilisers or feeding stuffs to bear an indication of origin on sale when the total quantity sold does not exceed 14 lbs. in weight.

5. Goods to which this Order applies shall bear the indication of origin herein provided on exposure for sale wholesale whether or not the person so exposing the goods is a wholesale dealer.

6. This Order shall come into force at the expiration of three months from the date hereof.

7.—(a) This Order may be cited as the Merchandise Marks (Imported Goods) No. 7 Order, 1931.

(b) The Interpretation Act 1889(a) shall apply to the interpretation of this Order as it applies to the interpretation of an Act of Parliament.

M. P. A. HANKEY.

(a) 52–3 Vict. c. 63.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Refractometric Studies on Fruit Juices.** H. Eckart. (*Z. Unters. Lebensm.*, 1931, 61, 346–353.)—The following values of  $n_D$  at 16° to 20° C. are given for the freshly-prepared juices during the season July–October, 1930 (*cf.* ANALYST, 1926, 51, 40):—Wild raspberry (sp. gr. 1.0440) 1.3480, (sp. gr. 1.039) 1.3462, (sp. gr. 1.025) 1.3422; garden raspberry (sp. gr. 1.0400) 1.3448; wild strawberry (sp. gr. 1.0390) 1.3460; garden strawberry (sp. gr. 1.052) 1.3439; red currant (sp. gr. 1.0410) 1.3471; white currant (sp. gr. 1.0444) 1.3475; black currant 1.3548; gooseberry 1.3503; bilberry 1.3408; blackberry 1.3412; cranberry 1.3460 and 1.3595; Sicilian lemon (sp. gr. 1.0340) 1.3438; Sicilian orange (sp. gr. 1.0520) 1.3519; acid cherry (sp. gr. 1.0640) 1.3561; sweet cherry (sp. gr. 1.0850) 1.3629; Italian grape 1.3645; Cornelius cherry 1.3702; elderberry 1.3551. The values vary over only a small range for juices made on successive days over a period of 1 month, and may be taken as characteristic for the season. Rejected juices had values 0.005 low, and addition of 250 c.c. of water to 250 c.c. of wild raspberry juice lowered  $n_D$  from 1.3473 to 1.339 (to 1.3452 for addition of 25 c.c. to 250 c.c. of juice). Readings taken every 2½ days for a month on the same stored juice showed a fall in  $n_D$  of 0.017 to 0.003 at the end of the period, and indicate the influence of auto-fermentation on storage. The Zeiss hand-refractometer designed for sugar control is recommended, and may be adapted to give direct readings of the dry solids.

J. G.



**Quartz Crystals in Honey.** F. E. Nottbohm and F. Lucius. (*Z. Unters. Lebensm.*, 1931, 61, 320-321.)—Particles of sand in honey may be due to mechanical inclusion or may be carried in the pollen or nectar. The possibility of the deposition of undigestible siliceous material by the bees suggested by Elser (*id.*, 1930, 60, 332) is discussed, and Elser's observations are criticised in that the hydrochloric acid-insoluble portion of the residue from the honey, after extraction with water, is regarded as silica, instead of the acid-insoluble portion of the ash. The authors find only 0.01 per cent. of acid-insoluble ash (corresponding with about 0.005 mgrm. of silica from one bee), and cannot regard the visible sediment, obtained by Elser on allowing the honey to stand, as quartz crystals. J. G.

**Iodimetric Determination of Reducing Sugars in the Apple.** H. K. Archbold and E. M. Widdowson. (*Biochem J.*, 1931, 25, 101-116.)—The oxidation of glucose and fructose by alkaline iodine at 1° C. and methods of preparation of apple extracts for iodimetric determinations have been investigated. Glucose is quantitatively oxidised to gluconic acid in two hours at 1° C., and some oxidation of fructose also occurs. The theoretical amount (1.410 grm.) of iodine is reduced per 1 grm. of glucose. In mixtures of glucose and fructose the amount of iodine reduced per grm. of fructose increases from 0.013 grm. to 0.017 grm. as the ratio of fructose to glucose increases from 1:1 to 5:1, and then decreases slowly as this ratio is further increased. The value 0.017 can be used for determination of fructose and glucose in apples by combination of the iodimetric and copper reduction methods, since the ratio of fructose to glucose in the apple is about 4:1. The amounts of fructose and glucose are then calculated by solving the simultaneous equations used by Evans (*Ann. Bot.*, 1928, 42, 1): (1)  $C_1x - C_2y = \text{iodine value per 100 c.c. of solution}$ , and (2)  $K_1x - K_2y = \text{copper reducing power of 100 c.c. of solution}$ , where  $C_1$  and  $C_2$  are the grms. of iodine reduced per grm. of glucose and fructose, respectively, and  $K_1$  and  $K_2$  the grms. of cuprous oxide formed per grm. of glucose and fructose at the dilution used. The new and more accurate values 1.410 and 0.017 are used for  $C_1$  and  $C_2$ . The presence of oxidisable material, other than sugar, makes it necessary to clear the apple extracts before carrying out iodimetric determinations. Basic lead acetate, with either sodium phosphate ( $\text{Na}_2\text{HPO}_4$ ) or potassium oxalate as the de-leading agent, was found to give satisfactory results with extracts prepared from mature apples. The loss of sugar during clearing, and the slight action of iodine on potassium oxalate, make corrections necessary for both the copper-reducing value and the iodine value, if oxalate is used to remove the lead. If sodium phosphate is used there is no loss of sugar, but the cleared solution is yellow and still contains some non-sugar substance oxidisable by iodine. The solution can be decolorised by boiling with charcoal and satisfactory results obtained. Copper reduction determinations can be carried out on the yellow solution. With very immature apples coloured solutions are also obtained when potassium oxalate is used, and these solutions cannot be boiled, as an increase in the iodine value occurs owing to some action of oxalate on the sugars. Sodium phosphate has, therefore, been adopted as the de-leading agent for routine work.

P. H. P.

**Selective Fermentation. Alcoholic Fermentation of Mixtures of Glucose and Fructose by Brewer's and Sauterne Yeasts.** R. H. Hopkins. (*Biochem. J.*, 1931, **25**, 245-255.)—Experiments by the author on selective fermentation of mixtures of glucose and fructose by brewer's and Sauterne yeasts gave the following results:—The factor  $K_{G/F}$  for brewer's yeast was not appreciably influenced by the relative proportions of glucose and fructose in the original solution. The factor was not influenced by modification of the saccharase activity of brewer's yeast by the method of Willstätter, Lowry and Schneider (*Z. Physiol. Chem.*, 1925, **146**, 158; **150**, 287). The saccharase of Sauterne yeast resembled that of brewer's yeast in that it was inhibited more strongly by fructose than by glucose. The addition of saccharase to a mixture of glucose and fructose undergoing fermentation by zymin (brewer's) did not affect the selective fermentation of the two sugars. The factor  $K_{G/F}$  for brewer's yeast decreased with rise in temperature of fermentation, whilst that for Sauterne yeast increased. Brewer's yeast ferments glucose faster than fructose in separate solutions when the concentration of sugar is less than 1 per cent. Sauterne yeast ferments fructose faster than glucose in separate solutions at all concentrations up to 10 per cent., but especially at low concentrations. The muta-rotation of partly fermented solutions of the sugars is for both sugars by brewer's yeast in the positive, and by Sauterne yeast in the negative direction. The hypothesis is now advanced that brewer's yeast, and presumably most yeasts, are specific for that form of fructose which is present in small proportion, but which increases with temperature, possibly a  $\gamma$ -form, whereas Sauterne yeasts are specific for the normal form. If this hypothesis should prove correct, the problem of the selective fermentation of these sugars by various yeasts resolves itself into finding an explanation for the fact that brewer's and most yeasts are specific for certain hexoses, namely, those derived directly from maltose, sucrose, etc., and Sauterne yeasts for normal fructose as it occurs in grapes. P. H. P.

**Composition of Commercial Palm Oils. II. The Fatty Acids and Component Glycerides of some Palm Oils of High Free Acidity.** T. P. Hilditch and E. E. Jones. (*J. Soc. Chem. Ind.*, 1931, **50**, 171-176T.)—The previous communication dealt with samples of palm oils of relatively low acidity (*ANALYST*, 1930, **55**, 701), and similar analyses of the mixed fatty acids of other four palm oils of much higher acidity have now been made. With increasing free acidity the determination of the saturated acids (mainly myristic) becomes more difficult, owing to the presence of small amounts of decomposition products, the boiling point of which is about 35° to 100° C. at 1 mm., and although the greater part of the myristic acid is usually retained in the "solid" acids (which were not appreciably contaminated with rancidity products), yet the determination is not so close as that of palmitic, stearic or oleic acid. The neutral fats were prepared by adding saturated sodium carbonate solution to the ethereal solution of the fat, and, after settling without shaking, running off and repeating the addition until the mixture was alkaline to phenolphthalein, after which the next addition of sodium carbonate solution was gently shaken with the fat and the final addition

vigorously shaken. Residual soap was washed out and small amounts of neutral fats carried down by the soap were recovered by extraction with ether. The estimated composition of the four oils, omitting unsaponifiable matter, was:

		Myristic. Per Cent.	Palmitic. Per Cent.	Stearic. Per Cent.	Oleic. Per Cent.	Linoleic. Per Cent.
Bonny Old Calabar.						
Crude	..	4.1	40.1	4.4	41.5	9.9
Neutralised	..	2.5	40.8	4.3	42.5	9.9
Benin.						
Crude	..	4.5	37.5	4.2	47.3	6.5
Neutralised	..	4.9	39.1	2.3	45.5	8.2
Niger.						
Crude	..	5.9	39.3	2.2	42.7	9.9
Neutralised	..	3.2	36.0	4.1	46.9	9.8
Drewin "Gold Coast."						
Crude	..	2.2	35.3	5.2	52.3	5.0
Neutralised	..	3.0	33.6	5.3	52.3	5.8

It would seem that hydrolysis caused by rancidity proceeds, on the whole, non-selectively, the only exception being the Niger oil, and this may be due to some accidental cause. Considering the eight palm oils together, they are divisible into two classes; the Drewin (Gold Coast) oils containing 35 per cent. or less of palmitic and 50 to 52 per cent. oleic acid, and the remainder about 40 per cent. palmitic and 40 to 45 oleic. Similarly, the total percentage of saturated acids for the Gold Coast oils is about 42, and that of the other types 46 to 48.5, and the association ratio is 0.8 for the former and 1 for the others. Possibly the Drewin oils may be from different botanical species of oil palm. The extreme limits for stearic acid are 2.2 to 7.5 per cent. Such variation as there is in the component fatty acids of palm oil involves alteration both in the amount of palmitic acid and of the total acids of the  $C_{18}$  series, which is a point of difference from animal fats. The observed acetyl values of the neutralised oils varied from 11.0 to 26.3, except for the Niger oil, which had the value 51.4. These values for the high acid oils are low compared with those calculated from the free acid content, so that, for the most part, the fats have been completely hydrolysed to glycerol and fatty acids, the proportion of semi-hydrolysed mono- or diglycerides indicated by the acetyl values being comparatively small for Bonny Old Calabar, Benin and Drewin No. 2. The plantation oils of lowest free acidity had acetyl values of 11 to 12, indicating the presence of some mono- or diglycerides in the fresh fat as matured in the palm fruit, the amount corresponding with 4 to 8 per cent. of the oil expressed as diglyceride.

D. G. H.

**Quantitative Determination of Lecithin in some Fats.** E. Foyn. (*J. Pharm. Chim.*, 1931, 123, 465-474.)—The lecithin was determined by weighing about 5 grms. of the fat in a narrow glass tube, and pouring it, drop by drop, into

30 c.c. of fuming nitric acid in a 500 c.c. flask, finally dropping in the tube. Oxidation is brought about by warming for 30 minutes, after which the solution is evaporated on a water-bath, 6 c.c. of 10 per cent. nitric acid added to the residue, the whole mixed, placed on ice, and the solidified organic acids then washed on a filter paper with water acidulated with nitric acid. The liquid is filtered, concentrated to 10 c.c., transferred to a flask, and precipitation brought about by 30 c.c. of molybdic solution, and, after warming for 15 minutes, the liquid is left overnight, after which it is filtered, and the precipitate collected in a tared Gooch crucible and washed with a 5 per cent. solution of ammonium nitrate in 1 per cent. nitric acid until the excess of the molybdic solution is removed, when 60 per cent. alcohol is used to remove the ammonium nitrate, followed by 96 per cent. alcohol, and, finally, one washing with ether, after which the precipitate is dried and weighed. Taking the formula for lecithin as  $C_{44}H_{96}NPO_8$ , and that of the molybdic precipitate as  $(NH_4)_3PO_4 \cdot 12MoO_3$ , the lecithin is found by multiplying the weight of the precipitate by the factor 0.4287. For identification of the fatty material analysed the iodine value was determined by Margosches' method (*Die Jodzahlschnellmethode und die Ueberjodzahl der Fette*, Stuttgart, 1927), in which 0.1 to 0.12 grm. of liquid oil, or 0.2 to 0.4 of fat, is completely dissolved in 10 c.c. of absolute alcohol, and 25 c.c. of 0.2 *N* alcoholic iodine added, followed by 200 c.c. of warm water. After shaking and leaving for 10 minutes the excess of iodine is titrated. Figures so obtained were very similar to those given by the Hübl method. The proportions of lecithin and of nitrogen, and the iodine values of a large number of vegetable and animal fats are given, varying from 0.6 per cent. of lecithin in linseed oil to 0.04 in refined coconut oil, and 0.063 in cod-liver oil, to 0.017 in whale oil free from sediment. Lecithin is constantly present in small quantities in vegetable and animal fats, and appears to be dissociated from a more complex compound during the extraction of the fat; hence, two samples of a fat may not invariably give similar figures. The figures obtained by the above methods are comparable among themselves and approach the real values. The figures obtained for nitrogen were always higher than would be accounted for by the nitrogen in lecithin.

D. G. H.

**Fat and Phosphatid Contents of Cacao Beans.** B. Rewald and H. Christlieb. (*Chem. Ztg.*, 1931, 55, 393-394.)—In 17 samples of raw, dehusked cacao beans grown in various parts of the world, the moisture content varied from 3.8 to 6.6 per cent., the average being 5.2 per cent. The fat content of the water-free beans ranged from 52.7 to 61.9 per cent., the average value being 56.3 per cent. It is assumed that a lower percentage of fat than 50 per cent. should not occur normally. The phosphatid content showed limits of 0.02 and 0.256 per cent. (dry beans), the average being about 0.1 per cent. Owing to the fact that it simplifies production considerably, 0.1 to 0.5 per cent. of plant lecithin is often added during the manufacture of chocolate.

To determine the phosphatids, the powdered beans were extracted with a mixture of benzene (8 parts) and alcohol (2 parts), from 6 to 8 extractions being

found necessary. After evaporation of the solvent, the mixture of fat and phosphatids was taken up in ether and, after distillation of the ether, filtered until bright. From 6 to 8 grms. of the filtrate were treated in a Kjeldahl flask with 40 c.c. of Neumann's acid mixture, fuming nitric acid being added and heat applied, at first gently and later with a larger flame, until the liquid became perfectly clear and colourless. The cold liquid was diluted with 100 c.c. of water, and the phosphoric acid precipitated by means of ammonium molybdate. The next day the precipitate was collected on a Gooch crucible, washed with water until this showed a neutral reaction, returned to the Kjeldahl flask, and dissolved in excess of 0.25 *N* sodium hydroxide. The ammonia liberated was boiled off, and the cooled residual liquid titrated with 0.25 *N* sulphuric acid to determine the excess of alkali remaining. The basis of the calculation is the presence of 3.94 per cent. of phosphorus in lecithin (calculated from the formula for egg-yolk). Contrary to the statement of Fincke (*Die Kakaobutter und ihre Verfälschungen*, 1929), the whole of the phosphatids are completely extractable from cacao beans by the procedure described above.

T. H. P.

**Detection of Benzoic Acid as Methyl Ester.** L. Pick. (*Z. Uniers. Lebensm.*, 1931, 61, 358.)—A modified form of Röhrig's method (*id.*, 1908, 15, 27; *cf.* Fischer and Gruenert, *ANALYST*, 1909, 34, 394) avoids the difficulty due to the odour of substances natural to (*e.g.*) wine and meat products. Wine is extracted with ether or petroleum spirit in the presence of sulphuric acid, the extract made alkaline with sodium hydroxide and evaporated, and the residue gently warmed over the naked flame to remove any substances contributing aroma. A drop of dimethyl sulphate is added, and, on stirring for 1 minute, the characteristic smell of the methyl ester is produced from 10 mgrms. or more of benzoic acid. If necessary, the ester may be separated by extraction in ether in the manner described by Röhrig, when 1 mgrm. of benzoic acid is detectable. Meat products (*e.g.* 80 grms.) are minced, an equal weight of 2 per cent. sodium carbonate solution added, and the mixture strained through a cloth, the residue washed with water, and the extract filtered. Fatty acids are then removed by precipitation with calcium chloride or with lime, and the filtered liquid evaporated, acidified, and extracted with ether as with wines (sensitiveness 0.07 per cent.). Salicylic and *p*-hydroxybenzoic acids do not interfere, but in the presence of large amounts of chlorides the odour of methyl chloride is obtained.

J. G.

**Colour Reaction of some Drugs.** J. Sivadjian. (*J. Pharm. Chim.*, 1931, 123, 528–529.)—Uroselectan (sodium 5-iodo-2-hydroxy-pyridine acetate) gives a yellow precipitate with a 5 per cent. solution of ferric chloride, and a green-yellow coloration when treated with hydrogen peroxide containing 4 per cent. of sodium chloride. If 6 to 7 drops of bromine water are added to a few mgrms. of uroselectan dissolved in water, a Prussian blue coloration results, which, on addition of concentrated sulphuric acid, disappears and a precipitate forms. Ammonia brings back the blue colour, but if the ammonia is present in excess the

colour changes to pink. If plasmoquin and percaine are treated with tetrachloro-benzoquinone of chloranil in the presence of acetic acid, and the mixture boiled, a blue colour results with plasmoquin, and there is no change in colour with percaine. If, however, the reaction occurs in the presence of epichlorhydrin, acetic acid is not necessary, and percaine gives an emerald-green colour. If an ammoniacal solution of pyrocatechin is boiled with a little nitroprusside a cherry-red colour results.

D. G. H.

**Sivadjian's Colour Reaction for Identifying Ephedrin.** W. H. Hartung, F. Crossley and J. C. Munch. (*J. Pharm. Chim.*, 1931, 123, 474-478.)—Sivadjian's reaction for identifying ephedrin (*ANALYST*, 1930, 55, 763) was found to give less intense colours if the U.S.P. hydrogen peroxide was used in place of that from the Rhône-Poulenc works, but, if carried out as follows, intense and reproducible colours are obtained:—The substance is examined in a 0.1 *M* solution, and all amines are converted into their acid hydrochlorides. The solution (0.5 c.c.) is treated in a 10 × 100 mm. test tube with 4 c.c. of a 16 per cent. solution of sodium chloride, 0.45 c.c. of a 0.1 *N* alkali solution, and 6 drops of a 39 volume solution of hydrogen peroxide. After shaking, the tube is kept in boiling water for 2 minutes, when the colour reaches its maximum and remains so for about an hour at laboratory temperature. The colours given by 31 different substances are described according to the plates published by Mulliken in "Identification of Pure Organic Compounds," Vol. III. Sivadjian's colour reaction is not specific for the amino-alcohols of the ephedrine type; phenyl-ethanolamine gives a colour reaction, as also do amino-acetones, but the colorations with these are less intense than those with their corresponding alcohols. Aliphatic amino-alcohols or alcohols give no colorations, the presence of the aromatic nucleus appearing to be essential. The amino group intensifies the reaction, and the effect is greater if it is linked to the ring than to a side chain. Phenol gives a more intense reaction than aniline. The presence of a carboxyl group in a side chain inhibits the reaction. The reaction may thus be used to differentiate the isomeric toluidines, and the three amino-phenols.

D. G. H.

**Determination of Santonin in "Trochisci Santonini."** P. J. Claus. (*Pharm. Weekblad*, 1931, 68, 414-424.)—The Dutch official method and its modifications are described and criticised, and the following procedure suggested for mixtures of santonin and cocoa-butter (*e.g.* worm-tablets): The weighed sample (containing about 100 mgrms. of santonin) is dried at 105° C., ground, extracted for 5 hours in a Soxhlet apparatus with ether, and the extract filtered and evaporated, 0.5 grm. of paraffin-wax being added to the residue, to promote subsequent flocculation of the cocoa-butter. The residue is boiled with 50 grms. of alcohol (80° Gay-Lussac=sp. gr. 0.86416 at 15° C.) for 30 minutes under a reflux condenser, the cooled extract filtered on a paper wetted with the alcohol, and the residue re-extracted with 15 grms. of alcohol, both filtrates being collected in a separating funnel. They are then shaken with 15 grms. of petroleum spirit

(b.pt., 40° to 70° C.), the alcohol removed, the petroleum layer washed with 15 grms. of alcohol, and the total alcoholic liquid warmed for a short time to remove any petroleum spirit which otherwise interferes with the final titration. The mixture is then neutralised at room temperature to phenolphthalein with 0.1 *N* sodium hydroxide solution, 10 c.c. added in excess, and sodium santoninate produced by boiling under a reflux condenser for 20 minutes. The amount of alkali consumed is determined by titration in the cold with 0.1 *N* hydrochloric acid, and 1 c.c. 0.1 *N* sodium hydroxide solution  $\equiv$  24.6 mgrms. of santonin. Allowance should be made for the alkali used up in a blank experiment (0.3 c.c. for a pyrex flask). When 50 to 150 mgrms. of santonin were taken, the results were 2 per cent. low. If ether is replaced by chloroform, the results are high (about 5 mgrms.), since this solvent reacts with the alkali



J. G.

**Podophyllum Rhizome—American and Indian.** T. E. Wallis and S. Goldberg. (*Quart. J. Pharm.*, 1931, 4, 28–32.)—For determining the crude fibre in vegetable drugs, the following modification of the Dutch method, which is more efficient than the official method of the Ministry of Agriculture, is suggested: About 2 grms. of the material in No. 60 powder are treated with 50 c.c. of 10 per cent. nitric acid solution in a 200 c.c. flask, which is immersed during 15 minutes in a boiling water bath. The liquid is stirred meanwhile with a glass stirrer making 160 revolutions per minute. The contents of the flask are next transferred rapidly to a fine cloth strainer and filtered by suction. The residue is washed with 100 c.c. of boiling water, restored to the digestion flask, and heated and stirred as before with 50 c.c. of 2.5 per cent. sodium hydroxide solution. After a second filtration and washing, the residue is digested with 50 c.c. of 1 per cent. sulphuric acid solution, and again filtered and washed. The fibre is then placed in a weighed Berlin porous silica-bottomed crucible, dried to constant weight at 100° C., ashed, and the crucible again weighed. The difference between the last two weights gives the weight of crude fibre.

The crude fibre obtainable from American podophyllum (from *Podophyllum peltatum*, Linn.) by this procedure amounted to 5.35 per cent., whereas the Ministry of Agriculture process gave 7.5 per cent. The Indian drug (from *P. Emodi*, Wallich), the resin of which is about twice as active as that of the American variety, gave 7.2 and 10.5 per cent., respectively, by the two methods. When the drug was subjected to a preliminary exhaustion with 90 per cent. alcohol, the crude fibre obtained by the modified Dutch process from moisture-free American (Indian) podophyllum was 5.3 (6.7) per cent.

A commercial sample of powdered American podophyllum yielded 8.7 per cent. of crude fibre by the modified Dutch method, and was found to contain both Indian podophyllum and guaiacum wood (about 10 per cent.). A satisfactory reagent for identifying, and distinguishing between, the resins of the two drugs is 5 per cent. aqueous copper acetate solution, which gives a bright green coloration with an alcoholic (90 per cent.) solution of the resin of *P. peltatum*, and a brown

precipitate with a similar solution of the resin of *P. Emodi*. A detailed account of the anatomy of the two varieties of podophyllum, and of the structures by means of which they may be differentiated in the form of powder, is to be published shortly.

T. H. P.

**Studies on the Gums. II. Tragacanthin—The Soluble Constituent of Gum Tragacanth.** A. G. Norman. (*Biochem. J.*, 1931, 25, 200–204.)—A brief outline is given of the literature on the subject of gum tragacanth, which, like that of the gums in general, is fragmentary and confused. A name has never been given to the constituent of gum tragacanth soluble in water, and it is proposed to term it tragacanthin. On addition of water gum tragacanth swells enormously, since the water-insoluble form, bassorin, constituting 60–70 per cent. of the gum, gives a very bulky jelly. The tragacanthin may be separated by ordinary filtration in extreme dilution. The dilute filtrate obtained is concentrated under reduced pressure and treated with acid alcohol. The precipitate formed is filtered off, dissolved in water, and precipitated again. After several such reprecipitations the final dried product is a fine white powder very readily soluble in water. Tragacanthin is also separated effectively when a solution of the gum is first made alkaline, and then just slightly acid, and centrifuged. Uronic acid residues are found to be present and to constitute about one-half of the molecule. Arabinose was the only sugar found; no galactose could be detected. It seems likely that tragacanthin consists solely of uronic acid and arabinose, although it is only possible to account for 94 per cent. of the molecule in this way. This is not in agreement with the conclusion of O'Sullivan (*J. Chem. Soc.*, 1901, 79, 1164), who considered tragacanthin to be a complex poly-arabinon-trigalactan-geddic acid, yielding, on hydrolysis, arabinose, galactose and geddic acid, an isomer of arabic acid also obtained by him from certain constituents of gedda gum. Hydrolysis products were prepared, the analytical figures for which give rise to the suggestion that a portion of the arabinose is united to the uronic acid to form a resistant nucleus, and the residue attached by glucosidic linkage, and, therefore, easily removable.

P. H. P.

**Electrometric Studies of Complex-Formation. II. Tartrates of Bismuth.** C. Morton. (*Quart. J. Pharm.*, 1931, 4, 1–13.)—The results of electrometric titrations confirm the existence of the four sparingly soluble bismuth tartrates described by Corfield and Adams (*Year Book of Pharm.*, 1923, 576; 1924, 594), the ratio Bi:C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> having the values 1:1, 1:1.5, 1:2 and 1:2.5, respectively. These compounds, however, are not insoluble complex acids, but true salts of the weak acid with very weak base type. Their properties are satisfactorily accounted for on the assumptions that the 1:2.5- and 1:2-tartrates are acid salts of the formulae Bi<sub>2</sub>(C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>)<sub>3</sub>, 2C<sub>4</sub>H<sub>4</sub>O<sub>6</sub> and Bi<sub>2</sub>(C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>)<sub>3</sub>, C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>, respectively; that the 1:1.5-compound is the normal tartrate, Bi<sub>2</sub>(C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>)<sub>3</sub>; and that the 1:1-tartrate is a basic salt, Bi(OH)C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>. The "bismuth and sodium tartrates," obtained by dissolving the insoluble tartrates in sodium hydroxide solutions, are equilibrated mixtures of the soluble basic complex, 2Bi(OH)<sub>3</sub>, Bi(OH)C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>, with sodium



tartrate, there being no evidence of the existence of complex anions in the neutralised solutions. In the absence of complex formation, the precipitation of basic salts of bismuth by the addition of alkali commences at pH 1.7; precipitation is delayed by the presence of glycerol, but eventually a highly basic precipitate is obtained. It is suggested that the method of Corfield and Adams for preparing the normal or 1:1.5-compound, namely, the interaction of solid bismuth oxynitrate, tartaric acid, and water, be adopted as the standard procedure, and that "bismuth and sodium tartrate" be obtained by neutralisation of this product, with subsequent evaporation. Methods involving the use of alcohol or other precipitants are shown to be unsound.

T. H. P.

## Biochemical.

**Biochemistry of Aluminium. II. Excretion and Absorption of Aluminium in the Rat.** K. Mackenzie. (*Biochem. J.*, 1931, 25, 287-291.)—It has previously been shown by the author (*Biochem. J.*, 1930, 24, 1433), by balance experiments with pigs, that practically the whole of the aluminium provided as a supplement to the diet is voided in the faeces. With such animals there is a difficulty in devising a ration free from aluminium, and accordingly this work has been supplemented by experiments with rats, in which the ration could be built up by the use of highly purified foodstuffs. The three materials likely to contain aluminium, namely, the food, the urine and the faeces, were kept as separate as possible by means of specially devised individual glass jars (in which the rats were kept) and special food troughs. The experiments showed that rats receiving a diet containing aluminium excrete the aluminium entirely by way of the alimentary tract, and there is no clear evidence that any of the aluminium is excreted in the urine. No definite evidence of absorption of aluminium by the internal organs of such rats has been established. Therefore, aluminium plays no important part in the metabolism of the rat.

P. H. P.

**Vitamin A Content of Oats.** C. R. Meyer and R. A. Hetler. (*J. Agric. Res.*, 1931, 42, 501-506.)—Whole hull-less oats and acetone-extracted oat oil were given to rats depleted of vitamin A as the sole source of that vitamin in their diet. Whole oats up to a level of 60 per cent. of the diet in no case cured ophthalmia, and death occurred before the eight-week test period was finished. A slight improvement in the eye conditions was noticed when oil at the high level of 30 and 50 per cent. of the diet was used, but growth was very slow or completely arrested, although the animals lived over the test period. It is concluded that a very small amount of vitamin A is present in oat oil. The rapid cure of ophthalmia and resumption of growth which occurred on adding cod-liver oil to the diet show that the bad effects were not due to any toxic effect of the oats or oat oil.

D. G. H.

**Spectrographic Data Concerning Vitamin A and Liver Oils.** R. A. Morton, I. M. Hellbron and A. Thompson. (*Biochem. J.*, 1931, 25, 20-29.)—This work was done in order to place on record a number of spectroscopic observations on liver oils and concentrates. The data are primarily concerned with

the more precise description of tests for vitamin *A*, in particular with the ultra-violet absorption and the blue colour test. In the second place spectrographic methods have been utilised for a more penetrating investigation of the wider problem of the constituents of liver oils and concentrates. In this way it was hoped to gain some insight into the sequence of changes culminating in the synthesis of fat-soluble vitamins or pro-vitamins in nature. Although it is too early to estimate to what extent this hope has been realised, a completely fresh aspect of the chemistry of vitamin-bearing oils emerges from the discovery of new and highly characteristic absorption spectra, which cannot be identified with the properties of the major constituents of liver oils. Precise spectroscopic data regarding the vitamin *A* ultra-violet absorption band are recorded. The vitamin *A* band at  $328\mu\mu$  is in a high degree continuous, and is found to be free from fine structure. Nearly all cod-liver oils exhibit selective absorption in the region  $260\text{--}295\mu\mu$ . Spectroscopic examination of the blue solutions obtained with antimony trichloride discloses, with cod-liver oils giving a clear ultra-violet band at  $328\mu\mu$ , a single sharp band at  $604\text{--}608\mu\mu$ . This class comprises the majority of pale medicinal cod-liver oils, and the parallelism between blue colour and ultra-violet absorption is well marked without the introduction of correction factors. With crude cod-liver oils of high potency additional selective absorption between  $565\text{--}585\mu\mu$  is frequently observed in the blue solution. The blue solutions given with concentrates have the main band at  $620\text{--}624\mu\mu$  (the intensity varying directly with the intensity of the ultra-violet band at  $328\mu\mu$  in the original material), and many of them show a less intense band at  $582\text{--}593\mu\mu$ . Vitamin *A* is decomposed on treatment with sodium ethoxide. Concentrates so treated yield acids characterised by a series of well-defined absorption bands with maxima near 394, 375, 350, 330, 316, 302, 282, 271 and  $260\mu\mu$ . Similarly absorbing acids are produced by the ordinary saponification process, but evidence is adduced showing that these acids cannot be ordinary fatty acids, and that they are not present as simple glycerides in the oil itself.

P. H. P.

**Spectrographic Data of Natural Fats and their Fatty Acids in Relation to Vitamin *A*.** A. E. Gillam, I. M. Heilbron, T. P. Hilditch, and R. A. Morton. (*Biochem. J.*, 1931, 25, 30–38).—A detailed investigation of the absorption spectra of natural fats and their related acids has been started, following the observation that vitamin *A* concentrates yield, on treatment with sodium ethoxide, acids of which the absorption spectra exhibit characteristic fine structure. Certain broad aspects of the problem are considered, and not only vitamin *A*-containing oils, but also typical vegetable fats have been examined. The absorption spectra of the total acids from cod-liver oil are characterised by sharp bands with heads at about 392, 375, 350, 330, 316, 302, 281, 270, 259 and  $235\mu\mu$ , and correspond closely with those of the selectively absorbing acids from the vitamin *A* concentrates described by Morton, Heilbron and Thompson (*Biochem. J.*, 1931, 25, 24). The absorption spectrum of a halibut-liver oil rich in vitamin *A* shows the characteristic vitamin band at  $328\mu\mu$ , and a second small band at about

**225 $\mu\mu$ .** The acids obtained from this oil on saponification reveal bands with heads at identically the same positions as those of the cod-liver oil acids. The absorption curve for butter acids (butter being chosen to represent a non-liver animal fat containing vitamin *A*) shows five definite maxima, indicating the presence of small quantities of substances having the same bands as those shown by the cod- and halibut-liver oil acids. The spectrum of butter-fat in chloroform solution shows distinct selective absorption at 322, 309, 284, 274 and 231 $\mu\mu$ ; these bands correspond with those of the acids in alcohol. The absorption spectra of the mixed acids from whale oil (a typical marine animal non-liver oil deficient in vitamin *A*) are shown to be qualitatively, and roughly quantitatively, comparable with the cod- or halibut-liver acids. The oil itself, like butter, also has selective absorption similar to that of the acids, but different in intensity. It is shown that, as with butter, the selective absorption of the whale oil is not exclusively due to hydrolytic products of the fat. The absorption spectra of the mixed fatty acids of a Thresher shark-liver oil, from which vitamin *A* is absent, show ill-defined maxima at 270, 280 and 315 $\mu\mu$ , and a prominent band at 230 $\mu\mu$ . The absorption spectra curves of olive oil and its mixed acids and cottonseed oil and its acids are shown. The curves differ markedly from those shown by the vitamin *A*-containing liver oils and the corresponding acids of the latter. The vegetable oils exhibit a triplet group of bands with heads at 260, 271 and 281 $\mu\mu$ , and indications of other absorption in the region 300–350 $\mu\mu$ , but the typical vitamin *A* band is absent. The curves for the corresponding mixed acids, somewhat surprisingly, show less fine structure between 260–280 $\mu\mu$  than those for the respective oils. From results obtained it is improbable that the recorded fine structure can be attributed to esters of highly unsaturated acids ("clupanodonic" type). The simplest explanation in consonance with all the facts so far observed is that under hydrolytic conditions a substance (or substances) accompanying vitamin *A* gives rise to acid decomposition products which display intense selective absorption. Sufficient of this acidic decomposition product is formed under the ordinary mild conditions of saponification to give rise to the observed spectra. That the acid decomposition products are in some way connected with the presence of vitamin *A* is indicated by the distinctly different absorption curves obtained with the acids from the non-vitamin *A*-containing olive or cottonseed oils. The fatty acids (or esters prepared therefrom) produced in the ordinary hydrolysis of vitamin *A*-containing liver oils, or of some other fatty oils from animals whose liver oils contain vitamin *A*, yield highly characteristic banded absorption spectra which are absent from the corresponding acids of vitamin *A*-free oils.

P. H. P.

**Colour Reactions of Sterols with Nitric Acid.** O. Rosenheim and R. K. Callow. (*Biochem. J.*, 1931, 25, 74–78.)—From a chance observation it was found that nitric acid gives characteristic colour reactions with certain sterols; a systematic study of the reaction was, therefore, made. The colours produced were found to be transient when pure colourless nitric acid was used, but more stable when a mixture of glacial acetic acid and nitric acid (1:4) was

employed. Finally, it was found that nitric acid which contained the acetates of certain metals gave fairly stable colour reactions. A solution of mercuric acetate in nitric acid ("mercury reagent") is recommended as a general reagent. For its preparation 25 grms. of mercuric acetate are dissolved in 100 c.c. of nitric acid (sp. gr. 1.42). Nitrous acid interferes with the colour reactions, and it is necessary to decolorise the solution with a few crystals of urea. When this precaution is used the reagent apparently keeps indefinitely. The reaction is carried out by the addition of an equal volume of the reagent to a chloroform solution of the sterol, and immediate shaking. On account of the high specific gravity of the mercury reagent, the mixture separates rapidly into an upper coloured chloroform layer and a lower, usually colourless, layer of the reagent. A red colour with the mercury reagent indicates the presence of the  $\Delta^{1,2}$  (or  $\Delta^{1,13}$ ) linkage in sterols; red colours are given by *allo*-cholesterol, *allo*-sitosterol, cholesterolene,  $\psi$ -cholestene and  $\beta$ -cholesterol. The tint of the red colours varies somewhat with different sterols, and is orange, carmine red or magenta according to concentration, but all the solutions show selective absorption in the region of  $500\mu\mu$ . A greenish-blue colour, following a transient pink, is given by ergosterol in dilute solutions (a marked reaction showing even with 0.01 mgrm. in 1 c.c. of chloroform), whilst a yellow colour results when concentrated solutions of ergosterol are used. The latter reaction serves as a useful index for the purity of ergosterol, since oxidised or otherwise changed specimens give, finally, an intense green reaction under the above conditions. A gentian-blue colour is given by products containing vitamin A (certain liver oils and the "unsaponifiable" of cod-liver oil). Only those sterols can give colour reactions with nitric acid or with the mercury reagent which possess either the  $\Delta^{1,2}$  (or  $\Delta^{1,13}$ ) linkage or the unknown etheroid linkages of the ergosterol molecule.

P. H. P.

**Conversion of Carotene into Vitamin A by Fowls.** N. S. Capper, J. M. W. McKibbin and J. H. Prentice. (*Biochem. J.*, 1931, 25, 265-274.)—It has now been established with a fair degree of certainty by various investigators that carotene possesses vitamin A activity; also that, in the rat, carotene is converted into the "classical" vitamin A of liver oils. It seemed desirable, before any generalisations could be made, that similar experiments should be carried out with animals differing widely from the rat, and the fowl was chosen for the work which is described. Chickens were successfully reared to maturity on a synthetic vitamin A-free diet to which either carotene or cod-liver oil concentrate was added. The carotene was not stored in the liver unchanged, but was converted into vitamin A characterised by the blue colour given with antimony trichloride (absorption band  $610-630\mu\mu$ ), and the presence of an absorption band at  $325\mu\mu$ . The beaks and shanks of chickens, which had become colourless through the absence of carotenoids from the diet, did not become more yellow when carotene was added to it. The poultry disease, known as visceral gout, would appear to be related to vitamin A deficiency, and to be curable by the administration either of carotene or of cod-liver oil. It is shown that the fowl, as well as the rat, can

convert carotene into vitamin *A*; the vitamin *A* requirements of the fowl are higher than those of the rat, weight for weight, and the liver oil of the hen is normally very much richer in vitamin *A* than is cod-liver oil. These experiments support the general theory that, in animals, carotene behaves as a precursor of vitamin *A*. The result of recent work on the relation of carotene to vitamin *A* makes it clear that biological tests alone cannot distinguish between carotene and the "classical" vitamin *A*, and it would seem probable that vitamin *A* is a product of animal synthesis, and ultimately owes its origin entirely to carotene. Land animals can obtain carotene from vegetable matter, and Ahmad (*Biochem. J.*, 1930, 24, 860) has shown that carotene in diatoms is probably the source of the vitamin *A* of fish-liver oils.

P. H. P.

## Bacteriological.

### Laboratory Tests on the Durability of Philippine Woods against Fungi.

O. A. Reinking. (*Philippine J. Sci.*, 1931, 45, 77-91.)—Results are given of tests made on 15 kinds of Philippine woods and on Southern yellow pine from the United States, using 5 named species of fungi and 9 unnamed cultures of wood-destroying hymenomycetes isolated from various species of local timber. Wide-mouthed 2-litre Erlenmeyer flasks were prepared with layers of culture blocks,  $2 \times 2 \times 5$  cm., of different non-durable woods, alternated with layers of similar test-blocks of the wood to be examined. Pads of wet cotton-wool were placed at the bottom and top of the pile, and the plugged flasks sterilised and, when cool, inoculated with vigorous bean cultures of the fungi. After the flasks had been kept at room temperature for periods of 7 to 28 months, the character of the fungus growth, the effect on the test and culture blocks, and the weights of the test blocks after oven-drying, were determined.

The loss in weight during 15 months varied from a very small amount to 60 per cent. for the woods examined, and the general order of durability agreed fairly well with published data concerning the service of the woods under practical conditions. It is generally assumed that the resistance of a wood to attack by fungus is determined largely by toxic substances present, but, with highly resinous woods, the action of such substances is undoubtedly supplemented by a water-proofing effect of the resinous materials. The effective toxic constituents in wood vary widely, the tannin groups being important in many species, essential oils in many conifers, and alkaloids in certain other species.

T. H. P.

## Toxicological and Forensic.

"Ginger Paralysis." (*Brit. Med. J.*, 1931, 322.)—An epidemic of paralysis, which occurred last year in Tennessee as the result of drinking adulterated ginger extract, and was the cause of several deaths, has now been found by the United States Public Health Service to have been caused by tri-ortho-cresyl phosphate, which is used as a cheap adulterant of ginger extract. Physiological experiments

on monkeys showed that the drug was harmless when given by the mouth in large doses, but produced typical paralysis, when injected subcutaneously, both in monkeys and in other animals. Apparently, tricresyl phosphate is not absorbed from the alimentary canal of monkeys, and the erratic incidence of the paralysis in those who drank the adulterated ginger extract points to considerable differences in the extent of its absorption by human beings.

## Organic Analysis.

**Use of Benzylic Potassium Hydroxide for the Determination of Acetyl Groups in Substituted Acetamides.** S. Sabetay and J. Sivadjan. (*J. Pharm. Chim.*, 1931, 123, 530-531.)—The acetyl groups may be determined in substituted acetamides, such as stovarsol, phenacetin, etc., by adding 25 c.c. of 0.5 *N* benzylic potassium hydroxide to 0.5 to 0.6 gm. of the substances under examination, and boiling under a reflux condenser, together with a blank, for half-an-hour. After cooling, 20 c.c. of neutral ethyl alcohol are added, and the excess of hydroxide is titrated with 0.5 *N* hydrochloric acid, with phenolphthalein as indicator.

D. G. H.

**Action of Iodine Monochloride on Cholesterol. Determination of the Iodine Value.** H. Werner. (*Z. Unters. Lebensm.*, 1931, 61, 321-337.)—The iodine value of cholesterol was determined under varying experimental conditions by the following methods:—(a) Winkler (*id.*, 1922, 43, 201; ANALYST, 1925, 50, 523). Bromine is liberated by acidification of a mixture of potassium bromide and bromate. (b) Rosenmund and Kuhnenn's pyridine sulphate dibromide method (*id.*, 1924, 49, 105). (c) Kaufmann (*Z. Unters. Lebensm.*, 1926, 51, 5). Sodium tribromide is formed by the action of bromine on a saturated solution of sodium bromide in methyl alcohol. (d) The Hanus, Hübl and Wijs iodine methods. The results are given in terms of the halogen consumed, expressed as a percentage of the amount theoretically absorbed. They show that the bromimetric methods give more consistent results than the iodimetric methods, (b) and (c) being preferred, whilst (c) has an additional advantage in that the reagent is more stable. For quantities of 0.1 to 0.4 gm. of cholesterol values of 100 per cent. were obtained after 5 to 45 minutes (109 per cent. after 24 hours) with (b), and after 20 to 150 minutes with (c); (a) gave high results. Of the methods (d), that of Wijs gave the highest results (*e.g.* 207 per cent. with 0.1 gm. after 15 minutes). It is considered that deviations between the iodimetric methods are greater than would be expected when other unsaturated substances (*e.g.* fats and oils) are used, and are due to substitution of the cholesterol molecule to varying extents by the halogen. The sodium thiosulphate back-titrations were therefore carried out in a neutral medium (carbon tetrachloride), and the halogen acid (HX) determined by titration with 0.1 *N* sodium hydroxide solution to phenolphthalein, till a red colour, stable on shaking for 1 minute, was obtained. The acidity (*A*) was then expressed as a percentage of the halogen consumption (*e.g.* according to the general type of reaction  $-\text{CH}_2-\text{CH}_2-+2\text{X} \rightarrow \text{CHX}-\text{CH}_2-+\text{HX}$ ). The results from the

action of iodine monochloride on cholesterol and dihydrocholesterol and their acetates, and on cholesten and cholestan indicate that substitution, as well as saturation, takes place in the iodimetric methods used for cholesterol, the halogen absorption being relatively small, and  $A$  being about 50 per cent. for saturated compounds such as cholestan and dihydrocholesterol acetate. J. G.

**New Compounds produced during the Hydrogenation of Fish-Oils.** S. Ueno and R. Yamasaki. (*J. Soc. Chem. Ind. Japan*, 1931, 34, 151B.)—Three kilos. of the evil-smelling volatile matter from a hardened fish oil were shaken with ether and saturated sodium bisulphite, 55 grms. of recrystallised scaly crystals being obtained. On decomposition with sodium carbonate solution and extraction with ether, 22 grms. of a pale yellow liquid were obtained. Most of this distilled at 130–170° C. at 25 mm. pressure, had a solidifying point of –6° C., and contained 78.04 per cent. of carbon and 13.12 per cent. of hydrogen, corresponding with the empirical formula  $C_{12}H_{24}O$ . Since the melting points of the aldehydes found and their derived acids are much higher than those of the corresponding normal compounds, it was considered that the evil smelling compounds examined were isoaliphatic aldehydes. R. F. I.

**Laboratory Bleaching Technique for Fatty Oils.** J. T. R. Andrews and R. G. Folzenlogen. (*J. Oil and Fat Ind.*, 1931, 8, 183–185.)—In order to put laboratory and works bleaching on to a more comparable basis, it is suggested that the official A.O.C.S. method be modified to include control of the moisture content of the oil. It was found that in the laboratory test on a 300 grm. sample of oil, 3 per cent. of earth with 1 per cent. of water was very nearly equal in bleaching power to 6.0 per cent. of earth without water (the amount recommended in the official laboratory test), and this amount was the optimum. With such moisture control that the added water was about 1 per cent. of the weight of oil taken, laboratory bleaching with English earth was equal to, or better in efficiency than works bleaching. These conclusions were reached from experimental bleaches on works refined cottonseed oil, but some work on refined tallow and coconut oil gave similar results. The optimum moisture for works-scale bleaching is about 0.1 to 0.3 per cent., and under ordinary conditions approximately this amount of water is present. If the laboratory bleaching is carried out below 100° C., no benefit is derived from added water, but from 100° C. improvement is seen up to a maximum at about 135° C. D. G. H.

**Fur Dyes, Their Oxidation and Identification on the Fibre.** R. B. Forster and C. Soyka. (*J. Soc. Dyers and Col.*, 1931, 47, 99–109.)—This subject was investigated by Cox (*ANALYST*, 1929, 54, 694), whose paper was published while the authors' work was in progress. Two methods, which work well with such skins as rabbit, skunk, sable, beaver, and sheepskins, are now given for the removal of dye bases: (1) Five to ten grms. of the fibre, removed from the pelt, are extracted with petroleum spirit (ligroin) or ether (0.717) in a Soxhlet or indigo extraction apparatus, the extract being evaporated to dryness with 0.5 to 1 c.c.

of *N* hydrochloric acid in a vacuum on a water-bath. The residue is digested with 50 c.c. of 0.1 *N* hydrochloric acid, and the liquid cooled, filtered through several thicknesses of filter-paper to remove the fats, and tested for the bases. (2) The degreased fibre is digested on a water-bath for at least 5 hours with 50 c.c. of 0.1 *N* hydrochloric acid, which is then cooled, filtered, and tested.

If the fur base is present in sufficient quantity, it is sometimes possible to extract and identify it. The hydrochloric acid extract is made alkaline with sodium carbonate in the case of an amine, or neutral in the case of an aminophenol, and extracted with a suitable solvent, *e.g.* benzene or ether (0.717). A number of precipitation and colour tests are given for para- and meta- phenylenediamines, meta-toluylenediamine, ortho-, meta- and para-aminophenols, amidol, metol, pyrogallol, para-aminodimethyl-aniline, 2:4-diaminophenetole, para-aminodiphenylamine, *p*:*p'*-diamino-diphenylamine, and Bandrowski's base in various dilutions.

The following are the reactions at dilution of 1:10,000, using (1) aniline hydrochloride and ferric chloride (indamine reaction), (2) nitrous acid, (3) nitrous acid + R-salt, (4) sodium hypochlorite, (5) sodium acetate and *N*/20 diazobenzene chloride, (6) sodium acetate and *N*/20 nitrodiazobenzene chloride:

	1.	2.	3.	4.	5.	6.
<i>p</i> -Phenylenediamine	Green-blue	Very pale yellow	Red	White ppt. yellow solution on boiling	Dirty choc. ppt.	Yellow
<i>m</i> - " "	Choc. brown at 1:5000	Brown	Pink-brown	White ppt.	Yellow ppt.	Yellow ppt.
<i>m</i> -Toluylenediamine	—	Yellow	Pale scarlet	—	Brown-orange ppt.	Brown-orange ppt.
<i>o</i> -Aminophenol	Red-brown	Greenish-yellow	—	Brown	Brown ppt.	Brown ppt.
<i>m</i> - " "	—	Colourless	—	—	Yellow ppt.	Yellow ppt.
<i>p</i> - " "	Pink	Colourless	—	—	Faint brown ppt.	Yellow
Amidol .. ..	Cherry red	Orange	—	Orange-yellow	Brown	Brown
Metol .. ..	Pink-purple	Colourless	—	Colourless → yellow	Pale yellow	Yellow ppt.
Pyrogallol .. ..	Greenish-yellow	Yellow	—	Pale yellow	Yellow ppt.	Yellow
<i>p</i> -Aminodimethylaniline	Pink	Pink-yellow	—	Cherry-red	Cherry-red	Cherry-red-yellow
2:4-Diaminophenetole	Pale pink	Yellow	Cherry-red	Brown-yellow	Orange	Orange
<i>p</i> -Aminodiphenylamine 1:50,000 → brown → green	Pink	Orange red	—	Pale yellow	Pink-brown	Pink-brown
<i>p</i> : <i>p'</i> -Diaminodiphenylamine	Green-blue	Green	Colourless → blue	Green-blue → pink	Greenish blue	Pale green
Bandrowski's base ..	Brown → green → blue	Brown	—	—	—	—



Details of other dilutions and directions for making up the reagents are given.

Experiments on the oxidation of fur bases on the fibre have been made with meta- and para-phenylenediamines, the oxidation being carried out with hydrogen peroxide in presence of alum-tanned sheepskin, both mordanted and unmordanted. In all cases unoxidised base was found on the fibre, the extent of the oxidation being influenced by the nature of the mordant used and by the *pH* value of the dye liquor. A list of patents (all German) on the use of organic bases for fur dyeing is included.

T. H. P.

**Determination of Silk in Silk Fabrics.** D. Ongaro. (*Giorn. Chim. Ind. Appl.*, 1931, 13, 159-162.)—The Kjeldahl method is not only lengthy but, when applied to the determination of silk in fabrics, requires the previous removal of nitrogenous dyestuffs. Van Slyke's method gives uncertain results, since the hydrolysis of the silk proteins is influenced by extraneous matters present in the fabrics, and artificial melanins or humin substances are always formed to some extent. The author's results show that the aminic nitrogen of fibroin may be rapidly and accurately determined by oxidising the fibroin by means of alkaline permanganate and ascertaining the amount of ammonia formed. The percentage of nitrogen thus found agreed closely with that of the total nitrogen present. For a series of 10 samples of the fibroin, the ratio between the quantities of aminic nitrogen and total fibroin varied from 1:6.26 to 1:6.32, the mean percentage of aminic nitrogen being thus 15.89.

With a silk fabric, the procedure is as follows:—The fabric is dried at 105-110° C. for two hours or, if very exact results are required, until constant in weight. About 0.3 gm. of the fabric is rapidly weighed in a closed weighing-bottle and transferred to a boiling flask (about 1 litre), where it is boiled until dissolved (a few seconds) with 50 c.c. of 20 per cent. sodium hydroxide solution. Tap-water (150 c.c.) through which air, previously filtered through dilute sulphuric acid, has been passed for 2 hours (this prevents bumping during the subsequent distillation) is then added and the liquid is heated almost to boiling point. About 1 gm. of potassium permanganate per 0.15 gm. of fabric taken is next introduced and the flask is at once connected, through a spray-trap, with a condenser as for a Kjeldahl distillation. The liquid is boiled until it has given about 150 c.c. of distillate, which is collected in 50 c.c. of 0.2 *N* sulphuric acid, the whole being then titrated with 0.2 *N* alkali in presence of methyl red (0.02 gm. dissolved in 100 c.c. of boiling distilled water and the solution filtered). If *A* c.c. of the 0.2 *N* acid have been neutralised by the ammonia distilled over, the weight of pure silk in the fabric taken will be  $A \times 0.0028016 \times 6.292$ .

T. H. P.

## Inorganic Analysis.

**Bromimetric Determination of Ammoniacal Nitrogen, I.** B. Levy. (*Z. anal. Chem.*, 1931, 84, 98-106.)—The disadvantages of the determination of ammonia in its salts by oxidation with hypobromite and iodimetric determination

of the excess of this can be avoided if bromine water is substituted for hypobromite and the oxidation is carried out in presence of bicarbonate. The process given, which is suitable for determining from 0.006 to 0.02 grm. of ammonia in the form of any of its ordinary salts, is as follows: To one of two equal portions of the solution are added 15 c.c. of saturated sodium bicarbonate solution, and 0.067 *N* bromine solution (1.8 c.c. of bromine, 120 grms. of potassium bromide, 1 litre of water) is run in until there is an excess of about 4 to 5 c.c., as shown by the yellow colour of the liquid; this gives the approximate quantity of bromine required. For the actual determination, the other portion of the solution, contained in a glass-stoppered flask, is treated with the above quantity of the bromine solution; 15 c.c. of saturated sodium bicarbonate are added, the flask stoppered and kept for 5 minutes; 20 c.c. of potassium iodide solution (5 per cent.) and 20 c.c. of 2 *N* sulphuric acid are added (the latter cautiously down the side of the flask). The solution is kept for 3 to 5 minutes, and the liberated iodine titrated with 0.033 *N* thiosulphate solution, with starch as indicator. The same quantity of bromine solution is standardised by adding the same quantities of reagents and titrating with the thiosulphate. The calculation\* of the result can be made from the equation for the oxidation reaction



It is stated that the process can be used for the determination of nitrogen after the Kjeldahl digestion process, the acid being neutralised by sodium bicarbonate.  
S. G. C.

\* *Note by Abstractor.*—The arithmetical formula given for the calculation is not clear.

**Bromimetric Determination of Ammoniacal Nitrogen, II.** H. Tschepelewsky and S. Posdniakowa. (*Z. anal. Chem.*, 1931, **84**, 106–118.)—It is shown that the oxidation of ammonia to nitrogen by bromine is quantitative between *pH* 7.5 and *pH* 9.5, thus confirming Levy's process (*cf.* preceding abstract), in which sodium bicarbonate solution is used as the reaction medium. In strongly alkaline solution some nitrite is formed. In using Levy's process for the analysis of commercial ammonium salts, an addition of some phosphate should be made to prevent the interference, with the iodimetric titration, of any iron which they may contain. These salts may also contain impurities of a reducing nature; it is recommended, if these impurities are suspected, to heat the sample for from 30 to 50 minutes with from 10 to 15 c.c. of concentrated sulphuric acid in a Kjeldahl flask before proceeding with the process.  
S. G. C.

**Determination of Zinc in Cadmium.** A. R. Powell. (*J. Inst. Metals*, 1930, **44**, 81–82.)—The metal is dissolved in hydrochloric acid and the solution evaporated nearly to dryness. When cold, it is treated with ammonia (5:1 water) until a clear solution is obtained, and enough solid potassium iodide stirred in to convert the cadmium chloride into iodide (2.5 grms. of potassium iodide per grm. of cadmium) and also to yield a 4 per cent. solution of the precipitant. The white

crystalline precipitate of tetramminecadmium iodide,  $\text{Cd}(\text{NH}_3)_4\text{I}_2$ , is left to settle, filtered on loose paper, and washed with a 4 per cent. solution of potassium iodide in 80 per cent. ammonia (wash-bottle with Bunsen valve). The excess of ammonia is expelled from the filtrate by evaporation, the acidity of the liquid adjusted to 10 per cent. of hydrochloric acid, and the minute amount of cadmium left in solution precipitated with hydrogen sulphide and filtered off. The zinc in the filtrate is determined by the usual methods (*cf.* ANALYST, 1925, 50, 18). W. R. S.

**Iodimetric Determination of Bromide Ions.** Z. Szabo. (*Z. anal. Chem.*, 1931, 84, 24–30.)—Bromide ions can be oxidised quantitatively to bromate ions by chlorine water in the presence of potassium bicarbonate, provided that a large excess of chlorine is employed. The following process is proposed for the determination of bromide in solution:—To the solution (from 5 to 20 c.c. containing from 0.1 to 20 mgrms. of bromide), contained in a 300 c.c. beaker, are added from 1 to 2 grms. of crystalline potassium bicarbonate and the necessary volume of chlorine water (0.1 mgrm. of bromide requires 30 mgrms. of chlorine, more bromide requires more chlorine up to the 370 mgrms. of chlorine required for 20 mgrms. of bromide). The solution is evaporated practically to dryness. The residue is dissolved in 100 c.c. of water, and 10 c.c. of phenol solution (5 per cent.) are added, with stirring (the phenol is required to remove the last traces of chlorine). After 5 minutes, 1 gm. of potassium iodide is added, the solution is acidified with 25 to 40 c.c. of dilute sulphuric acid (20 per cent.), and, after a further 5 minutes, the liberated iodine is titrated with 0.01 *N* or 0.05 *N* thiosulphate solution, with starch as indicator. The amount of bromide calculated from the thiosulphate used (on the basis of reduction of bromate to bromide) is subject to a slight correction which falls from  $-0.027$  mgrm. (for 0.1 mgrm. found) to zero (for 3.0 mgrm. found), rising proportionately to  $+0.37$  mgrm. (for 20.0 mgrm. found). Any iodide present with the bromide to be determined must be removed by acidifying the solution with sulphuric acid and boiling after addition of potassium nitrite. No reference is made to the determination of bromide in the presence of chloride.

S. G. C.

**Volumetric Determination and Separation of Ortho-, Pyro-, and Metaphosphoric Acids.** S. Aoyana. (*Z. anal. Chem.*, 1931, 84, 31–35.)—The author has revised his earlier process (*J. Pharm. Soc. Japan*, 1925, No. 520, 553; *Bull. Imperial Hygienic Laboratories*, 1926, 27, 131), and now proposes the following method in which the solution, rendered slightly alkaline to phenolphthalein, is precipitated with silver nitrate, and the acid liberated (according to the equation  $\text{Na}_2\text{HPO}_4 + 3\text{AgNO}_3 = \text{Ag}_3\text{PO}_4 + 2\text{NaNO}_3 + \text{HNO}_3$ ) neutralised with borax; the excess of silver in the solution is titrated, whence the amount of silver equivalent to the total phosphate is obtained; the precipitate of silver phosphate is decomposed with hydrogen sulphide and the liberated phosphoric acid is titrated with alkali to methyl orange and also to phenolphthalein; the amount of the three phosphoric acids can then be calculated. *Method*:—To two equal portions of the solution containing the mixture of ortho-, pyro-, and metaphosphoric acids or their alkali

salts, in amount approximately equivalent to from 10 to 20 c.c. of 0.1 *N* silver nitrate solution, is added 0.1 *N* alkali until the solution reacts slightly alkaline to phenolphthalein. A preliminary trial is now made by adding to one portion 25 c.c. of 0.1 *N* silver nitrate solution and titrating with 0.1 *M* borax solution, the amount of the latter required to render the solution slightly alkaline to litmus paper being noted. To the other portion are added 25 c.c. of 0.1 *N* silver nitrate solution, followed by 0.05 c.c. less than the quantity of borax solution previously found, and the solution is diluted with an equal volume of alcohol. The solution is filtered, the precipitate washed with 50 per cent. alcohol, and the filtrate is diluted with an equal volume of water and its silver content titrated by the Volhard method; the difference between this amount of silver (expressed as c.c. of 0.1 *N* silver nitrate solution) and the amount initially added is called *a*. The filter carrying the precipitate of silver phosphate is placed in a flask together with not more than 30 c.c. of water, and hydrogen sulphide passed into it for 15 minutes; the liquid is filtered into a flask, the filter being washed until the runnings are neutral. The filtrate is evaporated to 30 c.c. under reduced pressure at a temperature below 40° C., transferred to a beaker with not more than 20 c.c. of water, 45 c.c. of saturated sodium chloride solution are added, and the liquid is titrated with 0.1 *N* alkali to methyl orange; the volume of alkali solution used is called *b*. Finally, a further 55 c.c. of saturated sodium chloride solution are added to this same liquid, and the titration with alkali continued until the end-point to phenolphthalein is reached; the total number of c.c. of 0.1 *N* alkali thus used is called *c* (a "blank" titration of 55 c.c. of the sodium chloride solution with the alkali, to phenolphthalein, is deducted from *c*). The amounts of ortho-, pyro-, and metaphosphoric acids can be calculated from the following:

$$3x + 4y + z = a; \quad x + 2y + z = b; \quad 2x + 4y + z = c;$$

where *x* represents the number of c.c. of 0.1 *N* orthophosphoric acid; *y* of pyrophosphoric acid; and *z* of metaphosphoric acid. S. G. C.

**New Method for Dissolving Cassiterite.** S. Tamaru and N. Ando. (*Z. anal. Chem.*, 1931, **84**, 89-98.)—Many attempts are described in which finely-ground mixtures of cassiterite, lime and carbon are heated with a view to finding a method of treatment of cassiterite which would yield the tin in a form soluble in hydrochloric acid. In the experiments in which crucibles were used for the heating, more or less of the tin always remained insoluble. The only successful experiment, in which all the tin was ultimately obtained in solution, was one in which 0.3 gm. of cassiterite, 0.78 gm. of lime and 0.0138 gm. of charcoal (0.0126 gm. of carbon was required to combine with the oxygen in the tube used) were placed in a boat which was heated in the closed end of a quartz tube (36 cm. long, 2.1 cm. diameter, 120 c.c. volume) at 900° C. for 1 hour; the free end of the tube projected from the furnace and was closed by a rubber stopper. After the heating, the product had a sintered appearance and dissolved completely in dilute hydrochloric acid (1:1). It is stated (without any supporting data) that, in the reduction of cassiterite by heating with potassium cyanide, some tin is lost by volatilisation of stannous oxide. S. G. C.

**Measurement of Hydroxyl and Hydrosulphide Ions in Sodium Sulphide Solutions.** A. W. Goetz. (*J. Amer. Leather Chem. Assoc.*, 1931, 26, 234.)—The hydrogen electrode being unreliable in the presence of sulphides, the use of the antimony electrode is recommended. It is, however, essential to standardise its form. An antimony rod, 5 inches long and  $\frac{1}{4}$  inch in diameter, cast in a hot mould, quenched and surface-polished, was found best from the point of view of time and stability. The E.M.F. values for sodium sulphide, sodium hydrosulphide, sodium hydroxide, and sodium carbonate, as found by the antimony electrode, are plotted against the logs of their respective dilutions. To obtain the  $pH$  values corresponding with the E.M.F. values as found by the antimony electrode, the cube roots of the sodium hydroxide normalities were plotted against the corresponding OH-ion concentration. Then the OH-ion concentration for any sodium hydroxide normality being known, and its corresponding E.M.F., the OH-ion concentrations of solutions of the above salts can be obtained from the graph (log OH-ion concentration against the E.M.F. of sodium hydroxide taken with the antimony electrode).

The  $pH$  values at decinormal concentration are found to be as follows:— $Na_2S$ , 13.86;  $NaOH$ , 12.96;  $Na_2CO_3$ , 11.09.

The E.M.F. changes of both the antimony and the mercury and mercury sulphide electrodes with sodium sulphide solutions from 0.1  $N$  to 1.0  $N$  closely approximate to the Nernst equation  $E = RT/F \ln (H)^+$ . R. F. I.

## Microchemical.

### Quinoline as a Microchemical Reagent for some of the Heavy Metals.

J. M. Kovenman. (*Mikrochem.*, 1931, 9, 223–228.)—Many salts of the heavy metals give sparingly soluble complex or double salts with quinoline in the presence of halogen salts. The sensitiveness of the reactions used as tests depends on the order of mixing the various reagents. The usual procedure is to mix a drop of quinoline or quinoline hydrochloride with a drop of saturated potassium iodide solution, and then to add a drop of the acid solution under examination. *Bismuth* gives a dark brown or orange-yellow precipitate seen under the microscope as crosses, rectangles and parallelograms; 0.1–0.2 $\gamma$  of bismuth may be detected. *Antimony* salts give similar crystals; 0.2–0.3 $\gamma$  of antimony is detectable. *Tin* reacts only in more concentrated solutions (1:300 or 1:500), 3 $\gamma$  being detectable; the crystals are seen as long yellow needles and parallelograms. *Mercuric* chloride reacts in neutral solution, and, after rubbing with a glass rod, the crystals are seen as colourless needles and rosettes; 0.25 to 0.3 $\gamma$  of mercury is detectable. The reaction is slightly less sensitive in hydrochloric acid solution, and less sensitive still in nitric acid solution. *Cadmium* salts alone in acetic acid or sulphuric acid solution give no precipitate with quinoline, but do so when potassium iodide (or other halogen salt) is also present, when 0.15–0.1 $\gamma$  of cadmium is detectable. The crystals appear as fine needles under the microscope. *Copper* salts in 1:100 to 1:200 dilution give an amorphous precipitate with quinoline. In the presence of potassium iodide the reaction is more sensitive and is characteristic. In acid

solution a dark brown crystalline precipitate is formed, appearing as rhomboids and octagons under the microscope; 0.15 to 0.2γ of copper is detectable. When ammonium thiocyanate is used instead of potassium iodide in the test for copper a green crystalline precipitate is formed; in neutral solution 0.15γ of copper is detectable. Under the same conditions cadmium gives colourless crystals appearing as rectangles or parallelograms, and 1γ of cadmium is detectable. *Zinc* salts give a characteristic reaction with quinoline and potassium bromide. Large rhomboidal crystals are formed, and 0.5 to 0.6γ of zinc is detectable. Mercuric chloride gives somewhat similar crystals under the same conditions, but, unlike the zinc compound, the mercuric compound is soluble in excess of the reagent. *Lead* salts give a crystalline precipitate with quinoline and potassium iodide, and 0.1γ of lead is detectable. The reaction is less sensitive in neutral solution than in acid solution. In the presence of a small amount of an oxidising agent the acid reagent forms a sparingly soluble quinoline periodide, which is seen as long needle-shaped blue-green crystals. This reaction is used as a test for dichromates. A reagent for testing for iron, zinc, cobalt and cadmium salts is prepared from equal parts of quinoline and ammonium thiocyanate, to which dilute nitric acid is added, drop by drop, until the quinoline dissolves. With *iron* salts the reagent forms red crystals of varied form, and 0.3γ of iron is detectable. *Zinc* salts give colourless rhombs and hexagons, but the reaction is not very sensitive. *Cobalt* salts give blue crystals of varied forms. *Cadmium* gives the most characteristic reaction, the crystals being formed in bushy groups; 3γ of cadmium is detectable. The reagent must be freshly prepared or crystals of  $C_9H_7N.HCSN$  are formed and interfere with the tests. Drawings of the various crystals are given in the original.

J. W. B.

**Systematic Qualitative Analysis by means of Modern Drop Reactions.** C. J. van Nieuwenburg. (*Mikrochem.*, 1931, 9, 199.)—A systematic qualitative analysis (which has been used by the students in Delft University for a year) combines some of the older group separations with the modern drop or "spot" tests as identification reactions. Only half the time is required for an analysis, and the results are better. The system is devised for the analysis of any mixture of the following cations:—Ag,  $Hg_2^{++}$ , As, Sb, Sn,  $Hg^{++}$ , Pb, Cu, Cd, Bi, Al, Cr, Te, U, Ni, Co, Mn, Zn, Mg, Ba, Sr, Ca, K, Na, and  $NH_4^+$ ; and the anions:  $Cl^-$ ,  $Br^-$ ,  $I^-$ ,  $CN^-$ ,  $CNS^-$ ,  $SO_4^{--}$ ,  $SiF_6^{--}$ ,  $NO_2^-$ ,  $NO_3^-$ ,  $HCOO^-$ ,  $CH_3COO^-$ ,  $SO_3^{--}$ ,  $S_2O_3^{--}$ ,  $S^{--}$ ,  $CO_3^{--}$ ,  $BO_3^{--}$ ,  $PO_4^{---}$ , and  $SiO_3$ . The amount of sample used is 50 to 200 mgrms. The preliminary examination by the dry method is retained. In the group precipitated with hydrogen sulphide the sulphides of arsenic, antimony and tin are dissolved in 2 N potassium hydroxide (in which, contrary to expectation, mercuric sulphide is practically insoluble), after stannous salts have been oxidised to the stannic condition with bromine water. The hydroxides of aluminium, chromium, iron and uranium form the next group, and cobalt, nickel, manganese, and zinc are then precipitated with ammonium sulphide together. Magnesium is grouped with the alkaline earths by gently heating with ammonium carbonate,

after expelling the excess of ammonium salts, the alkalis remaining together in solution. The final identification tests can be carried out on a drop of the solution as follows:—*Silver*, with a solution of rhodanine (dimethyl-amino-benzylidene-rhodanine) gives a red colour, whilst mercury does not react in the presence of potassium cyanide. *Mercury* gives an intense blue colour with an alcoholic solution of diphenylcarbazine. The mercury solution should be neutralised with potassium hydroxide, but should not be alkaline. *Lead* hydroxide is oxidised to lead peroxide with alkaline hydrogen peroxide, which should then be removed, and the lead peroxide is detected by the blue colour with tetra-methyl-diamino-diphenyl-methane. Bismuth must first be removed by extraction with sulphuric acid. *Bismuth* is identified by catalysing the reduction of lead acetate by potassium stannate. *Copper* is identified by the formation of blue-violet crystals on adding zinc sulphate and ammonium-mercury thiocyanate. *Cadmium* is identified by the formation of the metal by heating with sodium carbonate and charcoal in a capillary tube, and the subsequent formation of the sulphide with sulphur. For *arsenic* the Gutzeit reaction is used. *Antimony* is reduced with tin in hydrochloric acid, or, better, is identified by means of rhodamine B, after oxidation with solid sodium nitrite (Feigl, *Qualitative Analyse mit Hilfe von Tüpfelreaktionen*). *Tin* in the stannous state turns the yellow colour of a dilute solution of cacotheline, a nitration product of brucine (Dryer, *Chem. News*, 1883, **48**, 257), brown-violet. *Aluminium* gives a red stain on filter paper impregnated with alizarine S; this is developed over ammonia fumes and is not affected by a large excess of *N*/5 acetic acid, but ferric salts interfere. For *iron* the usual thiocyanate test is used. *Chromium* is converted into the chromate, which in acid solution gives a bright violet colour with diphenylcarbazine solution. *Uranium* is detected by the usual ferrocyanide reaction. *Cobalt* solutions with three volumes of ethyl alcohol and some crystals of solid ammonium cyanide give an intense blue colour. Interference of iron is prevented by the use of tartaric acid. A second test is the red-brown stain on filter paper with  $\alpha$ -nitroso- $\beta$ -naphthol, which (unlike the iron and nickel colorations) is not affected by 2 *N* hydrochloric acid. *Nickel* gives a red precipitate with dimethylglyoxime in slightly ammoniacal solution, or (in the presence of cobalt) tartaric acid solution, when hydrogen peroxide and finally solid sodium carbonate are added. *Manganese* with benzidine acetate in the presence of dilute acetic acid gives a bright blue colour. When cobalt is present tartaric acid, or a tartrate must be added. Marshall and Walter's reaction (*Chem. News*, 1901, **83**, 76; **84**, 239), using phosphoric acid instead of sulphuric acid for the formation of permanganate, is also used. For *zinc* the same reaction as for copper is used. A suspension of *magnesium* hydroxide, carbonate or phosphate in an alkaline liquid, boiled with a few drops of Titan yellow (azidine yellow, 5G) is coloured violet-red. The ordinary macro-chemical separation is used for calcium, barium and strontium. *Potassium* is identified by the cobaltinitrite reaction, *sodium* with zinc uranyl acetate, and *ammonium* in the usual way. The old tests are used for all the anions except silicates, fluorides and sulphides, for which Feigl's tests are used. Solid *sulphides* catalytically accelerate the reaction of a dilute solution of sodium

azide with a solution of iodine in potassium iodide solution, when nitrogen is vigorously evolved. Soluble *silicic acid* is boiled with a few crystals of ammonium molybdate and a few drops of dilute nitric acid; on cooling, a few drops of benzidine acetate and solid sodium acetate are added, and a blue colour indicates silicic acid. Phosphoric acid also reacts, and chlorides interfere with the reaction. For mineral silicic acid the substance is heated with a little solid fluoride and a few drops of concentrated sulphuric acid in a lead crucible, covered with a cellophane plate on which is hanging a drop of water. The water reacts with the silicon fluoride, which is converted into soluble silicic acid which can then be tested as before. *Fluorides* are similarly identified by heating with ground quartz in the lead crucible and then testing for silicic acid in the drop. Fluorides not decomposed by sulphuric acid must first be fused with solid sodium hydroxide. J. W. B.

**Detection of Gold, Palladium and Silver with Dimethylamino-benzylidene Rhodanine.** F. Feigl, P. Krumholz and E. Rajmann. (*Mikrochem.*, 1931, 9, 165-173.)—Rhodanine was first used by Feigl (*ANALYST*, 1928, 53, 615) as a reagent for silver and mercury. As other precious metals also react, giving coloured products with the reagent, the procedure must be varied according to the metal that is to be detected. The tests may be carried out in a test tube, when the coloured product is extracted with a few drops of ether, and is clearly seen on the boundary of the two liquids, or else as "spot" tests, using either a "spot" plate or filter paper impregnated with the reagent, and then dried. 1. *Detection of silver in the presence of gold, platinum and palladium.*—A drop of the test solution is mixed on a spot plate with a drop of 10 per cent. potassium cyanide solution, and then a drop of the alcoholic solution of *p*-dimethylamino-benzylidene-rhodanine is added and acidified with a few drops of *N* nitric acid, when the silver cyanide gives a violet colour with the rhodanine. As little as 1 $\gamma$  silver in the presence of 1000 $\gamma$  of mercury, 4000 $\gamma$  of gold or 300 $\gamma$  of palladium or platinum may be detected; the limit of dilution of silver is 1:50,000. Copper should not be present. 2. *Detection of gold.*—Gold salts give a red-violet precipitate with rhodanine in neutral and slightly acid solutions. The smallest amount detectable is 4 $\gamma$  of gold in a concentration 1:500,000 in 0.1 *N* hydrochloric acid solution when the test is carried out in a small test tube. As a "spot" test on paper, the smallest amount detectable is 0.1 $\gamma$  of gold, in the same dilution. *Detection of palladium in the presence of other metals.*—Platinum reacts slowly with the reagent (only as Pt<sup>++</sup>), so that when the test is carried out on impregnated filter paper the violet coloration due to palladium is visible almost immediately, and the coloration due to platinum is formed later in the zone outside, owing to diffusion through the capillaries of the paper. In this way 0.038 $\gamma$  of palladium can be detected in the presence of 20,000 times the amount of platinum. In the presence of gold or iridium the solution is boiled before the test with an alkali nitrite in the presence of excess of calcium carbonate, when both metals are precipitated, but the palladium remains unchanged. Osmium, rhodium and ruthenium salts give no reaction in 0.05 to 0.1 per cent. solutions, so that, after suitable dilution, these metals do not interfere.



When silver is present it is made inactive by the formation of the complex salt  $K[AgBr_2]$ , by treating the test solution with excess of solid potassium bromide. When the test is carried out in a test tube 1 $\gamma$  of palladium can be detected in the presence of 10,000 $\gamma$  of silver in a dilution 1:1 million. When carried out as a "spot" test 0.06 $\gamma$  of palladium can be detected in the presence of 1000 times the amount of silver in the same dilution.

J. W. B.

**Determination of Potassium in Dilute Solutions.** M. Wrangel. (*Z. anal. Chem.*, 1930, 82, 224-230.)—Potassium is precipitated as the cobaltinitrite, in which the nitrous acid is determined colorimetrically with indol sulphonic acid. The method is used for the analysis of soil solutions, and is suitable for determining from 0.02 to 0.06 mgrm. of  $K_2O$ , with an error of 0.2 per cent. For each analysis 20 c.c. of soil solution are evaporated to dryness in a quartz dish, and then gently heated to drive off the ammonium salts. The cold residue is dissolved in exactly 2 c.c. of 3 per cent. acetic acid, and 1 c.c. of the solution is pipetted into a centrifuge tube, and 1 c.c. of Tisdall and Kramer's (*J. Biol. Chem.*, 1921, 46, 339) cobaltinitrite reagent is added, and the mixture is left for at least 3 hours. It is then centrifuged for 20 minutes, and after removing the supernatant liquid, it is washed and centrifuged thrice, each time with 2 c.c. of water. The precipitate is dissolved by gently heating with 5 c.c. of 0.1 *N* sodium hydroxide, and the solution is transferred to a 100 c.c. measuring cylinder. After diluting to 98 c.c., 1 c.c. of indol solution (0.15 grm. of indol dissolved in 10 c.c. of alcohol, and diluted to 100 c.c. with water) and 1 c.c. of sulphuric acid (1:1) are added. The maximum carmine-red colour is reached after 5 or 10 minutes. The standard solution contains sodium nitrite equivalent to 0.005 mgrm. of  $K_2O$  per c.c. It is prepared from 0.4902 grm. of silver nitrite and sodium chloride. The filtrate is diluted to 1 litre, and diluted a further ten times before use. Potash-free glass should be used.

J. W. B.

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## Reviews.

REPORTS OF THE PROGRESS OF APPLIED CHEMISTRY. Vol. XV for 1930. Issued by the Society of Chemical Industry. Pp. 745. Price, post free, 7s. 6d. to members, 12s. 6d. to non-members, of the Society.

Unlike the Annual Reports on the Progress of Chemistry, issued by the Chemical Society of London, the volume under review contains no special chapter on analytical chemistry. This branch is, however, by no means neglected, and, in a number of the articles, is considered under a separate heading.

As is bound to be the case in a compilation of this kind, in which different subjects are reported by different authors, there is some unevenness in the manner of treatment adopted. This is particularly noticeable when the chapters on the fermentation industries and foods, respectively, are compared. Each of these

chapters forms a most readable and complete contribution, but the former is written in discursive fashion and occupies 40 pages, whereas the report on foods is only just over one-half as long. It may be that, in order to keep the size and cost of the book within reasonable limits, each subject is allotted a certain space, but many members of our own Society would doubtless like to have a little more room found for an all-important matter like foods.

The effects on industry of the world-wide economic depression of 1930, with the accompanying general drop in the prices of commodities, are discussed by a number of the contributors. In some sections such effects have been severely felt, although, on the whole, the chemical industry does not appear to have suffered so badly as certain others.

Perusal of this volume indicates no slackening in the efforts to improve plant and processes or to discover new uses for chemical products. Under "General, Plant, and Machinery," reference is made to the principal developments in the fixed nitrogen and other chemical industries at home and abroad, and to work on heat transfer, general plant operations and equipment, etc. The chapter on "Fuel" deals with economic problems such as scientific marketing, and with carbonisation, coke ovens, coal cleaning, and gaseous fuel. A number of investigations on the origin and composition of coal are described, the growing opinion that coal is derived mainly from the lignin, and not from the cellulose of the original plant material, being expressed. The question of atmospheric pollution is considered, and a section on the analysis and testing of fuels is included. In the chapter on gas, tar, etc., the stability and prosperity of the gas industry are indicated, and the examination of gas and of the bye-products of the industry is discussed.

The section on mineral oils deals at length with the important developments made in connection with hydrogenation, cracking, and the manufacture of intermediates from petroleum. Fewer special results than usual are recorded under "Colouring Matters and Dyes," although the volume of research work did not fall below that of recent years; it is gratifying to note the increasing proportion of inventions patented by British manufacturers. In the textile industries, the most noteworthy advances made during 1930 have been, not through new discoveries and inventions, but rather in the way of economics and trade organisation. As regards bleaching, dyeing, printing, and finishing, research has been devoted mainly to artificial silk, and, owing largely to the efforts of the English textile research associations, such research in this country has been more intensive than, and ahead of, that on the Continent. Much interesting work, but nothing of outstanding moment, is noticed under "Acids, Alkalis, and Salts, etc.," "Refractories, Ceramics, and Cements," "Iron and Steel," "Non-Ferrous Metals," and "Electro-Chemical and Electro-Metallurgical Industries." Under "Glass" are given references to various methods for the analysis of silicates and glass-making materials. Under specified conditions, 8-hydroxyquinoline has been found a good precipitant for separating alumina from molybdenum, uranium,

vanadium, arsenic, and fluorine; a rapid modification of the Berzelius method of determining fluorine, and a microchemical procedure for analysing as little as 0.01 grm. of glass are described.

"Oils, Fats, and Waxes" includes copious references to the fundamental investigations of Hilditch and his collaborators, and reports various testing and analytical methods. Carbon disulphide and sulphur may be detected by treating a chloroform solution of the oil with a copper salt and hydroxylamine hydrochloride, a chocolate-coloured solution and precipitate being formed. The Committee of the Society of Leather Trades' Chemists has decided in favour of the Hanus method of determining iodine values. Work on "Resins and Solvents" has been concerned largely with synthetic products, and under "Cellulose Ester Varnishes and Enamels" attention is drawn to the increasing use of compounds of the more complicated mixed types. The position of rubber, a commodity dependent, for its consumption, almost entirely on the tyre manufacturer, is adequately discussed, and a considerable volume of research recorded. With regard to "Leather and Glue," much work has been expended on the preservation of hides and skins, on the sterilisation of anthrax-infected hides, as well as on tannery operations and materials generally. Although no marked changes have been made in the analysis of vegetable-tanned leather during the present century, recommendations have now been made by a committee of the Society of Leather Trades' Chemists for the standardisation of the procedure involved in such analysis.

The salient feature of the article on "Soils and Fertilisers" is the marked interest shown in the Russian work on the morphology and genetic classification of soils, which is now becoming better understood in other countries. Important results following on the application of X-ray analysis to clays are noted, and reveal the possibility of establishing characteristic micro-crystals in soil clay. The suitability of bromocresol green as an indicator for Kjeldahl titrations is pointed out, and various methods for determining citric-soluble phosphoric acid are given. The high proportions of sucrose indicated in French beets of the 1929-1930 campaign by the direct polarisation method are ascribed to the presence of raffinose and nitrogenous substances, although another opinion is that pectic substances, more easily dissolved by the hot aqueous digestion of the analytical method than in the technical diffusion, caused the high results. Valuable information is obtainable by the "polarographic" method of examining sugar, the presence of molasses at 0.00002 per cent. concentration in 5 per cent. sucrose solution being detectable. In the chapter on "The Fermentation Industries," the examination of vinegar is considered, and methods of determining the dry extract and of distinguishing between different grades of wine vinegar and synthetic and distilled vinegars are reported.

Under the heading "Foods," attention is drawn to the continued interest in examination for vitamin contents. A large amount of work has been carried out on milk and dairy products. Further results go to establish the value of the freezing point in the detection of adulteration of milk, and it has been found that the difference

between the values for genuine and watered fresh milks is maintained if the milks acidify spontaneously to the same (but not too marked) extent. Analysis of numerous normal milks confirms the substantial accuracy of the Vieth ratio, lactose:protein:ash=13:9:2, these figures holding also for condensed and dried milks. The compressibility of the crumb has been suggested as a measure of the staleness of bread, and the retardation of staling by the action of aldehydes is regarded as due to combination of the aldehyde with the albumins present. The acidity of flour, for which a limiting value is included in the Greek Government specification, is discussed. The examination of foodstuffs for preservatives has come in for a good deal of attention. The determination of boric acid has been exhaustively considered in *THE ANALYST*, and the use of benzidine in preference to iodine has been suggested for determining sulphur dioxide, for which also a simplification of the Monier-Williams method has been proposed. A useful review of the literature dealing with the preservation of food is noted. Diverse opinions exist concerning the value of formol titration for detecting adulteration of honey with artificial invert sugar, and the value of a determination of hydroxymethylfurfuraldehyde for this purpose has been considered. Incipient putrefaction in meat and fish may be judged from the content of ammonium salts, the critical value being about 0.02 per cent. as  $\text{NH}_3$ . The pH value appears to be of little value for fixing the age of eggs, as, for the white, it increases from 7.6 to 9.0 in 7 days, and then remains unchanged, whereas, for the yolk, it alters from 6.0 to only 6.2 during 10 weeks. Relatively large amounts of aluminium appear to be innocuous to the animal organism, and the quantities of aluminium dissolved from cooking vessels of this metal to be devoid of physiological significance. Examination of the semi-carbazones has been found of value in the identification of the components of flavouring materials.

Under "Sanitation and Water Purification," emphasis is laid on the increased efforts necessary to deal effectively with sewage and trade effluents owing to the growth of the population and the development of industry. Chapters on "Fine Chemicals, Medicinal Substances, and Essential Oils," "Photographic Materials and Processes," and "Explosives" are followed by Name and Subject Indexes.

These Annual Reports, which must surely be among the cheapest scientific publications in the English language, are known so well that no recommendation is needed. It may, however, be stated that Volume XV is well up to the standard of its predecessors. Few errors, even in spelling, are noticeable, but "Wedgewood" is given twice in the text, although the index has it "Wedgwood."

T. H. POPE.

**THE CHEMICAL INVESTIGATION OF PLANTS.** L. ROSENTHALER. Translated from the 3rd German Edition by SUDHAMOY GHOSH. London: Bell & Sons. 1930. Price 12s. 6d.

This translation of Dr. Rosenthaler's well-known treatise will be welcomed by all who are interested in the analysis of plant products. A short historical introduction, very unequal in its treatment of the various groups, opens the general

section. This includes preliminary qualitative tests for the most important groups of compounds, and some account of the methods of extraction and separation. From the point of view of logical arrangement it is a pity that these two chapters are separated by chapters dealing with alkaloids and glucosides, which might more appropriately have found a place elsewhere.

The bulk of the book is occupied by the special section, the 19 chapters of which deal with fats, waxes, resins, proteins, etc. These contain a great deal of useful matter, but here, again, the treatment must be regarded as unequal. The chapter on organic acids is adequate, and the reference to the microchemical methods of Klein and Werner is welcome. Resins, alkaloids and glucosides also receive fairly full treatment. The qualitative part of the chapter on carbohydrates is full, but less than a page is devoted to the estimation of sugars. The chapter on colouring matters opens with the statement, "The majority of the colouring substances occur as glucosides," and only the anthocyanins are dealt with.

Several omissions have been noted. In connection with the fats a series of 12 colour tests is given without mention either of the results or of the conclusions to be drawn. The convenient benzidine test finds no place in the list of oxidase reagents. No mention is made of the goldbeater's skin test for tannins. Lichenin is said to give a blue coloration with iodine.

In addition to references in the body of the work a bibliographical appendix with 177 entries is provided; of these, only 7 refer to sources in English. It is a serious fault that no reference is made to such important English books as Onslow's "Anthocyanins," Osborne's "Vegetable Proteins," and Carré's "Pectic Substances." It is to be presumed that workers who require to use a translation of Dr. Rosenthaler's book would prefer to be guided to further details in their own language where these are available.

M. SKENE.

**BIOASSAYS. A HANDBOOK OF QUANTITATIVE PHARMACOLOGY.** By JAMES C. MUNCH. Pp. x+958. London: Baillière, Tindall & Cox. 1931. Price 45s.

The word "bioassay" is a new one; and although it is certainly less unwieldy than the terms in general use, "physiological assay" and "assay by a biological method," it is doubtful whether it can rightly be regarded as synonymous with the sub-title of this book, "A Handbook of Quantitative Pharmacology."

The present volume comprises a much wider field than that implied by the title. It is a compilation of general information and of the results of research relating to physiological testing. It includes much chemistry and pharmacology. The task which the author undertook was one demanding very great perseverance and the expenditure of much time. Over 17,000 references have been tabulated; briefly to consult and abstract a large proportion of these original communications alone has required an immense amount of application. It should be added here that the bibliography, given as an appendix to successive chapters, constitutes a valuable and important feature. It occupies about one-quarter of the text, some 230 pages in all.

The first three chapters deal successively with the general subject of physiological assays, the experimental technique, and the interpretation of results. In the six chapters which follow, drugs are roughly classified according to their action, those that act upon the nervous, circulatory, respiratory and muscular systems being treated separately. One chapter is given to hormones, and another to special types of drugs, and vitamins.

As a compilation of classified observations relating to quantitative pharmacology, the volume will prove invaluable to those who require a ready book of reference relating to this field of knowledge. On the other hand, those needing guidance in the choice of a biological method of assay will wish that the author had made freer use of his critical faculty in selecting that which is likely to prove of practical value, and omitting much which is not. By adopting this course, he would have given the reader who wishes to carry out physiological assays greater help in choosing the methods suited to his needs. Indeed, the book is more in the nature of a general textbook than of one dealing with methods of analysis.

Those unacquainted with the present state of our knowledge must not suppose it to be possible in more than a few instances to undertake true quantitative evaluation of active principles by biological methods. The title might lead one to hope for a collection of select methods of analysis giving a detailed description of the best procedure. The book contains very little that comes up to such expectations, and, if truth be said, accurate biological methods of assay are of too recent introduction to make possible a textbook of select methods of assay relating to more than a few substances.

The book is a truly useful one, dealing with the steps that have been taken towards the evolution of satisfactory methods of assay. It is well produced, and contains a number of useful figures and plates, and some 240 tables of classified information. To one wishing to commence research in this field, it will be found to be teeming with suggestions, for it shows only too clearly that our knowledge of pharmacology, although so extensive, is still in a very elementary state.

F. H. CARR.

**THE VITAMINS.** By H. C. SHERMAN and S. L. SMITH. An American Chemical Society Monograph. Second Edition. New York: The Chemical Catalog Company, Inc. 1931. Price \$6.00.

The first edition of this book was published in 1922. It was obvious at once that further editions would be called for as knowledge of the vitamins became extended. The second edition fulfils the promise of the first, and all the more important researches on the subject published before the middle of 1930 have been considered. This has involved the re-writing of large sections of the book in order to get relevant facts in their right relationship; the new facts have not merely been added at the ends of the appropriate chapters.

The book has singularly few mis-statements and mis-interpretations, but the account of one investigation is inaccurate, and may be misleading when, for practical reasons, it is essential that there should be no misunderstanding. Hess

and Weinstock are reported as having conferred antirachitic potency on certain foodstuffs by ultra-violet irradiation in 1924 and 1925, whereas Steenbock and Black are reported as having achieved this result in 1925. Actually Steenbock's first work on this subject was published in 1924, some months before Hess published any work on it. The paper in which Steenbock described this work is cited in the bibliography.

It is regrettable, also, that Sherman and Smith have retained and used so extensively the definition for a "unit of vitamin A" which was proposed originally by them, and which was later adopted by the United States Pharmacopoeia, for so many workers have pointed out the possibility of variation in the value of this "unit" that it seems to be impossible to accept it for general use.

The book is remarkable also for its bibliography which occupies some 180 pages, about one-third of the total number. Vitamin workers will be grateful to the authors for the care with which this has been compiled. Indeed, the whole book forms a valuable work of reference, a copy of which every serious worker on the subject will wish to possess.

KATHARINE H. COWARD.

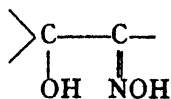
QUALITATIVE ANALYSE MIT HILFE VON TÜPFELREAKTIONEN. By Dr. FRITZ FEIGL. Pp. viii+387, with 12 figures in the text and 2 coloured plates. Leipzig: Akademische Verlags-gesellschaft M.B.H. 1931. Stitched R.M. 26.40; bound R.M. 28.

Dr. Feigl is the leading authority on the "spot" or "drop" test method of qualitative micro-analysis, in which a drop of solution (0.05 c.c.) is used for each test, and the reactions usually involve a colour change, either of the solution or by the formation of a coloured precipitate. The simplicity of most of the tests, and the rapidity with which they can be carried out, account for their increasing popularity, not only in the realms where micro-technique is essential, but also as an alternative to ordinary macro-procedure. Dr. Feigl's book is, therefore, particularly useful, in that it is the first book to cover the whole range of "spot" tests, not only with careful detail on the practical side, but also with considerable attention to all the underlying theory.

Most of the tests involve the use of organic reagents, many of which, in controlled conditions, give specific reactions with certain inorganic ions. The number of organic reagents is continually being augmented, and there is still a large field for research, since slight changes in the organic radicle may cause changes in the intensity of colour and solubility of the reaction produced. An advantage of organic reagents is that the large molecule often increases the visibility of the reaction product, and so decreases the amount recognisable by the test. Many tests may be rendered more sensitive by the use of an organic reagent—an example is the precipitation of magnesium as the hydroxide which is a very insensitive test, but in the presence of an organic dye stuff (*p*-nitrobenzene-azo- $\alpha$ -naphthol) which adsorbs magnesium hydroxide, as little as 0.24 $\gamma$  of magnesia in a dilution of 1 in 4 millions can be detected.

The presence of other substances may influence a reaction considerably, and

may sometimes completely mask it. Dr. Feigl, therefore, describes very thoroughly the theory of "masked" reactions, as well as the opposite effect, when the presence of other ions increases the sensitiveness of a test, and gives some well-chosen examples. There is a chapter on the theories and types of the different complex salts, as formed in "spot" tests, and the organic groups with specific reactions for certain inorganic ions, such as the NH-group for silver, and the



radicle for copper, are described in detail. There is an important chapter on the effect of various changes in grouping on the solubility of the reaction product in various solvents. The theoretical section ends with a chapter on capillarity as it applies to "spot" tests, for many tests are carried out on filter paper, when the capillaries of the filter paper play an important part.

The practical section of the book consists in descriptions of the tests for all the different inorganic ions, with full experimental details, which even include the method of preparation of the reagent when it is rare. Where a test is modified or masked by the presence of other ions, particulars are given. The smallest amount recognisable, and the limit of dilution, which are the terms always used by the author to define the sensitiveness of a test, are given for every test, and must have involved a large amount of work, since about 160 tests are given. Several methods have been devised for the systematic analysis of mixtures by the "spot" method, and the author describes four of these in detail.

The book ends with a number of valuable descriptions of the analytical procedure in the investigation of a number of special substances. The descriptions include tests for the purity of certain compounds, tests for copper in alloys, for lead in enamels, chromium in minerals, for mineral salts used in the preservation of wood, for traces of hydrogen sulphide in water, and many others.

The author is to be congratulated on having produced a book which, owing to its clear arrangement and care in descriptions of practical details, is excellently adapted for rapid laboratory reference, while the theoretical portion and the large number of references to contemporary literature render it valuable to all who are more than routine analysts.

JANET W. BROWN.

SULPHURIC ACID AND ITS MANUFACTURE. By H. A. AUDEN, M.Sc., Ph.D.  
London: Longmans, Green & Co. 1930. Price 16s.

The preface tells us that this book is designed, as the standard works on the subject are so costly and full of detail, "to help the student to grasp the fundamental problems associated with the subject, and by means of the accompanying references to enable him to consult the original articles when more detailed information is required."

It will certainly achieve its second object. The references are most numerous and complete, and, as a classified index to the literature of the subject, the book is



most valuable. Whether it will effectively attain its first object seems open to doubt; and certainly its effectiveness for that object could have been greatly enhanced by more attention to arrangement and style.

The book is divided into sixteen chapters:—1, Historical and Statistical; 2, Properties of Sulphuric Acid; 3, Handling and Transport; 4, Sulphuric Acid from Sulphates; 5, Sulphur and Sulphur Burners; 6, Pyrites Burners; 7, The Chamber Process; 8, Modifications of the Chamber Process; 9, The Gay Lussac Tower; 10, The Glover Tower; 11, Operation of Chambers; 12, Theory of the Chamber Process; 13, Purification of Sulphuric Acid; 14, Concentration of Sulphuric Acid; 15, The Contact Process; 16, Control of Contact Plant.

The descriptions of plant are for the most part very well done, though many of the diagrams are not very helpful, and might well have been omitted; and the accounts of the modifications of the original chamber process that have been proposed, of the working of chambers, and of the views that have been held by various workers both on the theory of the chamber process and on the advantages and disadvantages of different methods of working, are entered into very fully.

The student who has beforehand a general knowledge of the manufacture, and who seeks information on some particular point, will seldom consult the book in vain; but the beginner will find it difficult to acquire from it clear and adequate knowledge of the subject—chiefly because of faulty arrangement, but also through the frequent irrelevant interpolations and the style of the writing, here and there ungrammatical, and frequently involved and far from clear.

Chapter 5, for instance, is headed "Sulphur and Sulphur Burners"; but after some description of the occurrence and extraction of sulphur (in which, by the way, one might have expected some mention of Frasch and his discoveries), we come to spent oxide, zinc blende, and other sources of sulphur dioxide, with descriptions of burners devised to make use of them, after which we revert to sulphur, continuing (and, to some extent, repeating) the subject of nine pages earlier, and only now dealing with sulphur burners. And Chapter 7, "The Chamber Process," begins by discussing nitric acid and sodium nitrate, and the methods of introducing nitrous gases into the chambers, before any mention is made of the chambers themselves—thus it happens, too, that not only are the Gay Lussac and Glover towers mentioned, but influences on their working are discussed, many pages before the towers themselves are described. Many other instances of a similar lack of order could be cited.

On the second count it may be pointed out that in the Chapter on "Sulphuric Acid from Sulphates," several processes are described, the aim of which is the production of sulphates (ammonium sulphate, for example), not of sulphuric acid from them; and at the beginning of Chapter 7 reference is made, with a very elaborate, but not very illuminating diagram, to the manufacture of nitric acid from ammonia, in a place where the *source* of the nitric acid used is a matter of pure irrelevance. Similarly, the description, on p. 69, of Reich's method of determining sulphur dioxide in burner gas is broken into by an interpolation in its

middle of a paragraph on determining oxygen and total acidity; and on p. 149 the two parts of a statement, that 60 per cent. of the loss of nitre is due to one cause and 40 per cent. to others, are separated by another statement that the total loss is 2.68 parts of nitre per 100 of sulphur burnt. These instances are typical, and they make comprehension of the subjects treated much more difficult—for an ignorant learner, sometimes impossible.

With regard to the style, such sentences as "With the exception of greater ease in cleaning the shallow tray type, the Kessler pattern seems to have the advantage of greater output," are all too frequent; and redundancies like, "It melts, *when quite pure and free from water*, at 14.8°," or "By securing an inlet gas *so far freed from impurities as to be* chemically pure," only hinder the reader.

Careless writing is common—"A Study . . . are of interest"; "A knowledge . . . are helpful"; "Between each baffle"; "Much data"; "Dealt with in a four-compartment precipitator, each of which"; and such phrases as "Quite reasonably rapidly," "Somewhat cold," "Of the order of about," "Rather strictly," "Rather arbitrary," disfigure the pages frequently. The author is far from being alone, unfortunately, in his use of the misleading, not to say lying, adjective, "water-white" for colourless—but if water is white, what adjective are we to use to describe snow, milk, or chalk?

There are some errors of other kinds which should be noticed. There is surely something wrong in the dates on the fourth and fifth lines of p. 7; as early as 1862, on the Tyne, pyrites had displaced sulphur so far that 73,000 tons of pyrites were burnt, against 2000 tons of sulphur. It is a little difficult to see (p. 13) how Kohlrausch in 1898 could have re-examined and corrected results given by Knietzsch in 1901. On p. 16, *c* is given as the *required* mixture, but we are told how to make one part of *a*; the formula, for *c*, should read  $x = (b - c) / (b - a)$ . On p. 69 the mode of calculation is very badly put, and the formula is wrong: it should read  $(11.14 \times 100) / (m + 11) = \text{percentage of sulphur dioxide}$ . On p. 88 it is not the "heat of formation" of chamber acid which is reduced by using water-sprays instead of steam, but the total amount of heat produced in and introduced into the chamber, the argument being that the reduced amount lessens the need for cooling. The phrase (p. 178), "The current, about 620 kilowatts per 24 hours" is perhaps just a slip, but suggests a very hazy idea of the meaning of electrical units.

Some clerical or typographical errors occur; on p. 4, l. 9, *or* should be *and*; p. 42, l. 27, *for* should be *from*; p. 64, l. 23, *same* should be inserted before *low*; p. 67, l. 11, the final *s* of *temperatures* should be deleted; p. 75, the footnote to reference figure 3 is wanting; p. 88, l. 14, *tower* should be *lower*; p. 111, the diagram is lettered "*Glover*" instead of "*Gay Lussac*" tower; p. 126, l. 11, *ton* should be *lb.* (or, perhaps better, "20" in the previous line should be "44800"); p. 213, *channeling* is misspelt.

The book is admirable in its intention, and ought to serve a very useful purpose; and if I have dwelt more upon its defects than its excellences, this is from no desire

to decry it, but from an honest feeling of disappointment that it should fall short of the ideal the author has had in mind, and in the hope that a future edition may be called for, and opportunity afforded for the thorough revision that could enormously improve the book.

J. T. DUNN.

A MONOGRAPH OF VISCOMETRY. By GUY BARR, D.Sc. Oxford University Press; London: Humphrey Milford. 1931. Price 30s. net.

Dr. Barr's monograph has supplied a definite need in English literature for a comprehensive collection of methods of viscosity measurement and a critical study of their comparative values. For the most part, the subject of viscometry has been dealt with in rather diverse manners in text-books devoted to wide subjects, such as oil technology. The treatment of the subject in one volume is, therefore, more than welcome.

After a few historical notes Dr. Barr explains the theories of the viscous flow of liquids in tubes, describes the experiments of the pioneers of viscosity measurements, and deals fully with methods for the measurement of viscosity in absolute units. The Redwood, Saybolt and Engler viscometers are discussed under this heading, since their essential dimensions have been chosen so as to give results which can be directly translated in terms of absolute viscosity. This is rather an important finding, since viscometers of this type are often wrongly stigmatised as empirical.

Chapter V deals with capillary viscometers for routine work in which relative values are sufficient and in which liquids of known viscosity are accepted as standards. The precautions necessary in using such viscometers are carefully outlined, and it is emphasised that they can be made to give more accurate results than absolute viscometers in which experimental conditions require more elaborate precautions.

The subsequent chapters deal with viscous flow between parallel plates, capillary tube methods for gases, the falling sphere method and Stokes's law, rotational and oscillational viscometers, and, finally, miscellaneous methods which have been limited in their application, as, for example, the rolling sphere and air bubble methods. The last chapter discusses anomalous systems, such as colloidal suspensions, gels, etc., in which viscosity measurements are affected by other properties. In view of the difficulties of definition of "plastic solids" and "viscous fluids," this chapter might possibly have been amplified.

The book is well written, and the subject-matter is well arranged. Although Dr. Barr states that he has not attempted to give a full bibliography of the literature of viscometry, nevertheless the references given at the end of each chapter form a useful source of such information.

Both author and publishers are to be congratulated upon producing a valuable text-book upon a subject the treatment of which has hitherto been rather diffuse.

J. G. KING.

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

### NORTH OF ENGLAND SECTION.

THE Second Summer Meeting was held at the Queen's Hotel, Scarborough, from July 3rd to 6th, when forty-four were present, including several ladies. The President (Dr. J. T. Dunn) was accompanied by Mrs. Dunn, and the Hon. Secretary of the Society (Mr. F. W. F. Arnaud) by Mrs. Arnaud, and other members from the south included Mr. E. M. Hawkins, Mr. E. Hinks and Mr. A. Lucas.

On Saturday afternoon Dr. C. Ainsworth Mitchell (Editor of *THE ANALYST*) read a paper, illustrated by lantern slides, on "Documentary Evidence in Criminal Trials," and Mr. A. Lucas contributed to the discussion.

The Chairman (Mr. C. J. H. Stock) thanked the members of the Section and the members of the Society in general for their support, which had helped to make the Summer Meeting such an unqualified success. He recalled the fact that in former years such meetings were regularly held by the Society, and laid stress on their social value in enabling members to become better acquainted.

He then proposed the following resolution, which was carried unanimously:

"That the members of this Summer Meeting of the North of England Section of the Society of Public Analysts and Other Analytical Chemists desire to convey an expression of loyalty and goodwill to the Council and of devotion to its aims and interests. They wish also to express their sense of the honour paid to the Section by the presence at the meeting of officers and members of the parent body."

A vote of thanks to the Hon. Secretary of the Section (Mr. J. R. Stubbs) was proposed by the President and unanimously passed.

### Death.

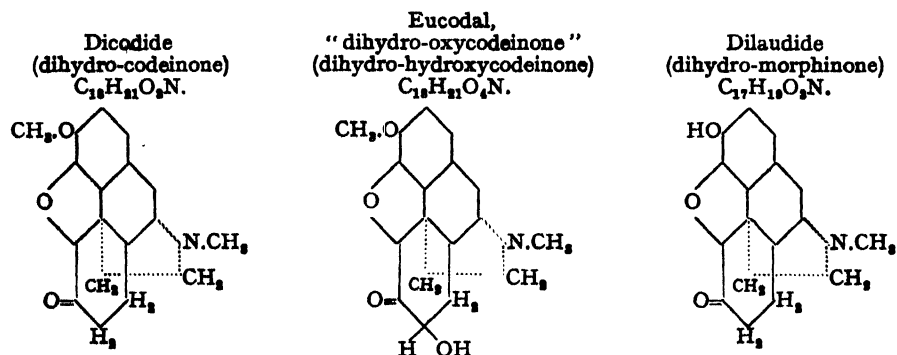
WITH deep regret we record the death of Mr. S. G. Agar (Official Analyst for the Island of Guernsey), on March 2nd, 1931.

# The Identification and Determination of Dicodide, Eucodal and Dilauidide.

By JOHN KING, F.I.C.

THE identification and determination of dicodide, eucodal and dilauidide have become important, owing to their recent inclusion in the list of drugs falling within the provisions of the Dangerous Drugs Act, 1920, Part III. Eucodal and dicodide are included under Statutory Rules and Orders No. 644 of Aug. 14, 1928, and dilauidide under No. 530 of June 13, 1930.

Their constitutions may be given as follows:



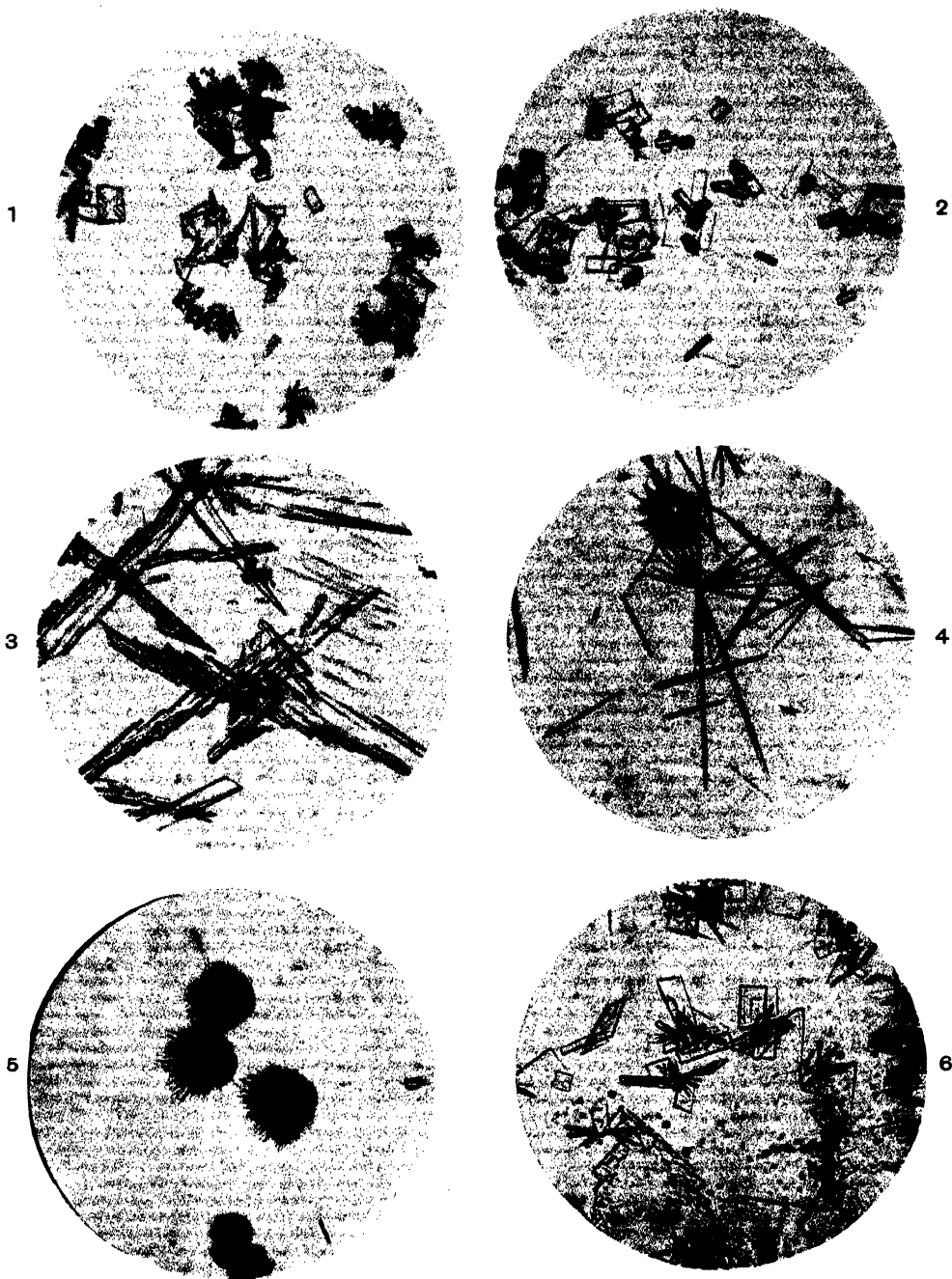
The structural formulae are based on Robinson's structure for codeinone (Gulland and Robinson, *J. Chem. Soc.*, 1923, 123, 980; *Mem. Proc. Manch. Lit. Phil. Soc.*, 1925, 69, 79), which Schopf's recent work (*Annalen*, 1927, 452, 211) has shown to be more probable than that of Wieland (*Ber.*, 1925, 58B, 2009), or of Freund and Speyer (*J. prakt. Chem.*, 1916, 94, 135; *Ber.*, 1906, 39, 844; 1924, 57, 1404).

These substances have been prepared from morphine, codeine or thebaine by suitable oxidation and reduction methods, and, as would be expected from their structures and derivation, they exhibit many similarities in properties both amongst themselves and with morphine and its derivatives. They are prescribed medicinally as hydrochlorides, bitartrates and phosphates. The solubility of the free bases in water at ordinary temperatures is very small, but increases rapidly with rise of temperature in a manner similar to that of morphine. The hydrochlorides are readily soluble in water, giving neutral solutions, the bitartrates being less soluble and giving acid solutions.

Dicodide and eucodal are precipitated by alkalis from solutions of their salts and are not soluble in excess of the reagents. The precipitates are crystalline, so that the bases may be identified microscopically. The crystalline forms of the bases and of some of their derivatives are shown on the accompanying photomicrographs. The bases may remain in super-saturated solution for a considerable







- |  |        |
|--|--------|
| (1) Dicodide (5 per cent.) and 7 per cent. ammonia.              | × 100. |
| (2) Dilauidide (5 per cent.) and 7 " " "                         | "      |
| (3) Eucodal (1 per cent.) and 7 " " "                            | "      |
| (4) Morphine (1 per cent.) and picrolonic acid.                  | "      |
| (5) Dilauidide (0.5 per cent.) and picrolonic acid (1 per cent.) | "      |
| (6) Dilauidide (10 per cent.) and palladous chloride.            | "      |





time, and it is generally necessary to scratch the microscope slide in order to induce crystallisation when employing a micro-crystalline test.

Dilaudide is precipitated from its salts by alkalis, and, as might be expected from the presence of a phenolic hydroxyl group, is soluble in excess of the reagents, its solubility in ammonia distinguishing it from morphine.

Eucodal is moderately soluble in petroleum spirit, dicodide sparingly soluble, and dilaudide insoluble. All three bases are moderately soluble in ether and readily soluble in chloroform, differing from morphine in these respects.

**MICRO-CRYSTALLINE TESTS.**—The bases are precipitated from solutions of their salts at concentrations of 1 per cent., or even less, by the following alkaloid reagents:—Mayer's reagent, Scheibler's reagent, Wagner's reagent, phosphomolybdic acid, silicotungstic acid, picrolonic acid and bromine water, and, at concentrations somewhat higher, by chromic acid, mercuric chloride and palladous chloride. These precipitates, with the following exceptions, are amorphous: Wagner's reagent gives a crystalline precipitate only with eucodal, which somewhat resembles that given by morphine, but is sufficiently characteristic to make a micro-crystalline test of value in identification. Picrolonic acid (1:4-dinitro-1-phenyl-3-methyl-5-pyrazolone) gives a crystalline precipitate only with dilaudide, its form resembling phenyl-lactosazone, and being of value in a micro-crystalline test. It is necessary to scratch the slide to induce crystallisation. The crystals given by morphine with picrolonic acid are readily distinguishable from those given by dilaudide. All three alkaloids give crystalline precipitates with a 1 per cent. solution of palladous chloride in dilute hydrochloric acid when the concentration of the alkaloid is 10 per cent. or over. They form circular clusters of plates or needles and resemble each other and the compound given by morphine. This micro-crystalline test should be carried out alongside known specimens under identical conditions, and should be used only as a confirmatory test.

The above, together with the crystalline form of the free bases, are the only micro-crystalline tests, as all other reagents tried have given amorphous precipitates.

**COLORIMETRIC TESTS.**—Marquis' reagent gives with each a bluish-violet colour, becoming red on standing. The colours given with the three alkaloids are not identical, nor are they quite the same as those given by morphine and heroin. In order to distinguish them, actual trials of known specimens must be made under conditions identical with those used with the substance under examination.

Iodic acid, in presence of dilute sulphuric acid, gives a yellowish-brown colour with dilaudide, as might be expected from its structural resemblance to morphine, but the colour is closer to yellow than that given by morphine. On adding ammonia a mahogany colour is given, and, on standing, this becomes distinctly more red, in contrast with morphine, which gradually assumes a dull brown colour. This test is quite characteristic for dilaudide.

When to 1 ml. of a dilute aqueous solution of a salt of these alkaloids 1 ml. each of 3 per cent. hydrogen peroxide and 7 per cent. ammonia solution are added,

followed by 1 drop of 10 per cent. copper sulphate solution, the following colours are given:—Dilauidide, brown to yellow; and morphine, brown to red. Eucodal and dicodide have no appreciable effect on the blue colour of the copper ammine. On adding a few drops of 10 per cent. potassium cyanide (which removes the colour of the copper ammine), the colours given are:—Morphine, red; dilauidide, yellow; eucodal and dicodide, very pale red. The test may be made extremely delicate for dilauidide by suitably reducing the amount of reagents added. As little as 1/20 mgrm. can be detected with certainty, and the test distinguishes dilauidide from all other alkaloids.

Radulescu's test (*Chem. Centrbl.*, 1906, 1, 1378) for morphine is also given by dilauidide, but not by dicodide or eucodal. The test is carried out as follows:—To a dilute aqueous solution of the alkaloid are added a few drops of dilute hydrochloric acid and sodium nitrite solution. After five minutes a few drops of strong ammonia solution are added, a brown colour indicating morphine or dilauidide. The test is extremely sensitive and will detect 1/50 mgrm.

Like many ketones, the three bases may be condensed with aldehydes in presence of alkalis (Lapworth, *J. Chem. Soc.*, 1911, 99, 1884). They condense readily with piperonal in alcoholic solution in presence of sodium hydroxide, giving yellow piperonylidenes. These cannot be obtained in crystalline form. The piperonylidenes give a brilliant violet colour with concentrated sulphuric acid, and this may be used as a confirmatory test for the alkaloids. They give precipitates with the usual alkaloid precipitants.

Fröhde's reagent gives with dilauidide a deep violet colour, changing on standing to intense blue. This resembles the colour change given by morphine and distinguishes these two alkaloids from eucodal and dicodide, which give no colour with the reagent.

Like morphine, dilauidide reduces potassium ferricyanide and gives Prussian blue with a mixed solution of ferric chloride and potassium ferricyanide. Eucodal and dicodide do not give this reaction.

All three alkaloids resemble morphine in giving a red colour when coupled with diazotised sulphanilic acid, and this test distinguishes the group from most other alkaloids.

Dicodide and dilauidide form oximes, unlike morphine, which has no ketonic group. The oxime of dicodide may be obtained by warming a solution of the hydrochloride in water with hydroxylamine hydrochloride, cooling and adding sodium hydroxide. It may be recrystallised from aqueous pyridine. The oxime of dilauidide may be prepared by heating as above and adding ammonia to the cooled solution. It should be recrystallised from alcohol. I have not been able to prepare the oxime of eucodal by the above methods, or by that of Lapworth (*loc. cit.*), and no reference to the oxime appears in the literature.

**SEPARATION AND DETERMINATION OF THE BASES.**—Where the bases occur in the pure state, they may be titrated, using as indicator methyl red, which gives

a well-defined end-point. Excess of  $N/10$  acid is added and the excess back-titrated with  $N/10$  alkali. Where they occur singly in the form of salts, the bases may be extracted from an ammoniacal solution by chloroform, and then titrated after removal of the solvent. The determination of mixtures is a matter of difficulty, and no one scheme is satisfactory for all cases.

The general scheme of H. C. Fuller described in his book, *The Qualitative Analysis of Medicinal Preparations*, 1920, has been in use in the Government Laboratory for some years, and is convenient in many cases. This scheme consists in extracting acid and neutral substances successively with petroleum spirit, ether and chloroform, and then basic substances (after addition of ammonia) with petroleum spirit, ether, chloroform, and, finally, a mixture of chloroform and alcohol. In this scheme, the three bases will appear in the separations after addition of ammonia in the following order:

- (1) *Petroleum Spirit*. Eucodal will be extracted. If present in large quantity, the three extractions given by Fuller may not be sufficient to remove the whole of the base, and some may appear in the ether fraction. A small amount of dicodide will also appear here.
- (2) *Ether*.<sup>\*</sup> Dicodide and dilaudide will be extracted; and if dilaudide is present in large quantity, it may not be completely extracted by three extractions, and will appear in the chloroform fraction.

Owing to the ease with which these three ketonic bases condense with aldehydes, ether free from aldehyde should be used. The bases extracted with impure ether are often yellow and non-crystalline.

- (3) *Chloroform*. Any of the three alkaloids remaining will be removed in this fraction.

If heroin, benzoyl morphine, or other esters of morphine are present, traces will appear in the ether fraction, the bulk being removed in the chloroform fraction.

Morphine, if present, will appear only in traces in these extractions. It is completely removed by extraction with a mixture of alcohol and chloroform (Nicholls, *ANALYST*, 1922, 47, 506). It may be determined either by titration or colorimetrically. Heroin or other esters of morphine, if present with dilaudide, may be hydrolysed to morphine, which may then be removed and determined in any convenient way.

The separation of dilaudide presents no difficulty in mixtures of dangerous drugs. The mixed bases should be extracted from an ammoniacal solution by a mixture of chloroform and alcohol, and the extract evaporated to dryness. The extract should be dissolved in dilute hydrochloric acid and the solution made alkaline with sodium hydroxide. A chloroform extraction will now remove only eucodal and dicodide, morphine and dilaudide remaining in the aqueous layer. This is saturated with ammonium sulphate and again extracted with chloroform, which removes dilaudide. Morphine remains in the aqueous layer and may be removed by a mixture of alcohol and chloroform.

Dilaudide may be determined colorimetrically by the iodate method or by the methods given later. In the presence of some coloured preparations, particularly those containing opium extracts, it is very difficult to obtain the alkaloid in a condition suitable for colorimetric determination. Extraction of the free base by lime water in the presence of freshly prepared free lime is often successful in reducing the amount of colour. The bases may be recovered from this solution by adding excess of ammonium sulphate and extracting with a mixture of alcohol and chloroform. In the case of opium preparations it may be impossible to eliminate the whole of the brown colour; this may often be matched, however, by that produced by a known amount of morphine or dilaudide acting on iodic acid. In this case the colours should be matched when the solutions are acid, as the addition of ammonia so alters the tint that matching is impossible. The amount of morphine or dilaudide equivalent to the initial colour of the preparation before the addition of iodate should be subtracted from that equivalent to the final colour, after adding the reagents to the test solution. The colour given in Radulescu's method is sometimes more convenient for matching than that given in the iodate method.

The directions given by Nicholls for the colorimetric determination of morphine (ANALYST, 1922, 47, 506) apply equally to the determination of dilaudide. Iodic acid needs approximately eight times as much dilaudide to produce a given intensity of colour as morphine in acid solution, and approximately five times as much in ammoniacal solution. This should be borne in mind when adjusting quantities for comparison of colours with standards, which should contain about 2 mgrms. of dilaudide per ml.

A more delicate method for the determination of dilaudide than the above is the copper and hydrogen peroxide test, which should be employed when only very small quantities of material are available. It has the added advantage of being specific for dilaudide. The solution for the test should contain approximately 0.1 mgrm. of dilaudide per ml. and should be neutral or slightly acid. To approximately 20 ml. of the solution are added 6 drops of 3 per cent. hydrogen peroxide, followed by 3 drops of 1 per cent. copper sulphate solution and 3 drops of 7 per cent. ammonia solution. After standing 5 minutes, 2 drops of 10 per cent. potassium cyanide solution are added, and comparisons made with standards similarly prepared. It is often convenient to employ small flat-bottomed specimen tubes which are carefully matched for size and colour of glass, instead of the usual Nessler tubes, which may be too large for the quantity of material available. As little as 5 ml. of a 0.01 per cent. solution may be used in this way.

Dilaudide may also be determined, when present in minute quantities, by Radulescu's method previously mentioned. The concentration of the alkaloid should be about  $1/20$  mgrm. per ml. for convenient colour matching.

Eucodal and dicodide may be partly separated from each other by extraction from ammoniacal solution with petroleum spirit, in which dicodide is only sparingly soluble. A satisfactory method for their quantitative separation has not yet been found.

**IDENTIFICATION.**—The specific rotations of the alkaloids may be used as a check both in identifying an alkaloid and in determining its quantity. If a narrow-bore polarimeter tube is used, an accurate determination may be carried out with as little as 0.1 gm. The tube which I have used is a 2 dcm. tube of approximately 4.5 mm. bore, holding about 3 ml. The readings are practically as exact as those obtained when using the usual pattern of tube, and are often a reliable guide to the identity of an alkaloid. It is found that a slight excess of hydrochloric acid may be added to the free bases in order to dissolve them, without the excess altering the specific rotation of the hydrochloride. When only 0.1 gm., or less, of a base is extracted, the procedure is to dry and weigh in the anhydrous form, dissolve in the minimum volume of *N*/2 hydrochloric acid, transfer to a 5 ml. graduated flask, make up to volume, and filter into the narrow-bore polarimeter tube.

The physical constants of the alkaloids are as follows:

Dicodide	—	M. pt. of base	.. ..	193–194° C.
		„ „ oxime	.. ..	265–266° C.
		[ $\alpha$ ] <sub>D</sub> of hydrochloride	.. ..	–143°
		[ $\alpha$ ] <sub>D</sub> „ bitartrate	.. ..	–79°
Eucodal	.. ..	M. pt. of base	.. ..	220° C.
		[ $\alpha$ ] <sub>D</sub> „ hydrochloride	.. ..	–136°
Dilaudide	—	M. pt. of base	.. ..	259–260° C.
		„ „ oxime	.. ..	231–233° C.
		[ $\alpha$ ] <sub>D</sub> of hydrochloride	.. ..	–132°

The specific rotations are calculated for the anhydrous salts in 2 per cent. aqueous solutions at 20° C.

**SEPARATION FROM COCAINE.**—The separation of cocaine from eucodal and dicodide presents some difficulty. It is readily extracted by petroleum spirit, and the three alkaloids are extracted in the same fraction in Fuller's scheme. Cocaine in this case should be tested for qualitatively, and, if present, hydrolysed to ecgonine by gently warming with dilute sodium hydroxide solution. Eucodal and dicodide can be extracted from this product, but ecgonine is not removed by any of the immiscible solvents and remains in the aqueous layer. It may then be removed and determined by adding sulphuric acid and phosphomolybdic acid, which precipitates the phosphomolybdate. This is washed with dilute sulphuric acid, filtered off, and boiled with barium carbonate. The mixture is filtered, the filtrate (containing free ecgonine) evaporated, the residue (the free base) dried and weighed. The equivalent weight of cocaine can be calculated.

I wish to thank the Government Chemist for permission to publish this paper, and Mr. J. F. Hirst and Mr. R. Stewart for the preparation of the photomicrographs.

GOVERNMENT LABORATORY,  
CLEMENT'S INN, STRAND, W.C.2.

## A New Method for the Detection of the Nitro-Group in some Organic Compounds.

By P. K. BOSE, D.Sc.

(Read at the Meeting, December 3, 1930.)

So far as I am aware, there is no characteristic colour reaction for aliphatic nitro-compounds. Although Victor Meyer's method (*Annalen*, 1875, 175, 93) may be utilised for the characterisation of primary and secondary nitro-paraffins containing less than seven carbon atoms in the molecule, it cannot indicate a tertiary nitro-group. Konowalow's reaction (*Ber.*, 1895, 28, 1851) suffers from a similar disadvantage, although, unlike Victor Meyer's reaction, it is given by higher nitro-paraffins (primary and secondary). Moreover, nitrous acid (as in V. Meyer's method) gives colour reactions with many other compounds of a different chemical nature (e.g. secondary amines, aromatic tertiary amines, phenols, etc.), and it can hardly be employed with advantage for the identification of nitro-paraffins.

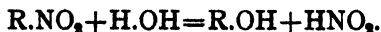
In the aromatic series, the well-known diazo test for amines is usually applied after reduction of the nitro-bodies in acid solution. But a similar behaviour is shown by hydrazo-, azo-, azoxy- and nitroso-compounds, which are also convertible into primary amines under identical conditions. Moreover, the diazo test is hardly suitable for showing the presence of a nitro-group in a primary nitro-amine, since the parent compound, as well as its reduction product, will obviously respond to the diazo test with almost equal facility. Mulliken and Barker's method (*Amer. Chem. J.*, 1899, 21, 271), which consists in the reduction of nitro-bodies to hydroxylamine derivatives, and subsequent identification of the latter by their reducing action upon silver nitrate, suffers from many of the aforesaid drawbacks, inasmuch as nitroso-, azo- and azoxy-compounds behave like nitro-compounds. The adaptation of Coupier's process for the production of magenta, as a test for an aromatic nitro-group, is equally unsuitable, since a positive reaction is given by many organic compounds containing oxygen directly linked to nitrogen, as also by iodo- and iodoso-benzene (Mulliken and Barker, *loc. cit.*).

References in this connection should be made to the colour reactions given by certain nitro-compounds, both aliphatic and aromatic, with unsaturated compounds, aromatic hydrocarbons, phenols and phenol ethers, bases and metallic salts (Werner, *Ber.*, 1909, 42, 4324; Graham and Macbeth, *J. Chem. Soc.*, 1921, 119, 1362; Will, *Ber.*, 1914, 47, 964; Harper and Macbeth, *J. Chem. Soc.*, 1915, 107, 87; Giua and Giua, *Gazzetta*, 1921, 51, 313; Sudborough and collaborators,

*J. Chem. Soc.*, 1911, 99, 209, and subsequent papers; Ciusa and Vecchiotti, *Atti. R. Accad. Lincei*, 1911, v, 20, I, 803; 20, II, 377; Goddard, *J. Chem. Soc.*, 1921, 119, 1161). These, however, are not general, and are not applicable to nitro-bodies as a class. Dimethylaniline (Walter, *Z. Farbenind.*, 1911, 10, 49) and aluminium bromide (Olivier, *Rec. trav. chim.*, 1918, 37, 241) give colour reactions with many aromatic nitro-compounds, and apparently these two reagents may be used with advantage for the characterisation of aromatic nitro-compounds.

Janovsky (*Ber.*, 1891, 24, 971) discovered that aromatic dinitro-compounds in acetone solution give characteristic colorations with aqueous potassium hydroxide, and this sensitive reaction has subsequently been extended by Bitto (*Annalen*, 1892, 269, 377), Reitzenstein and Stamm (*J. prakt. Chem.*, 1910, 81, 167) and by Rudolph (*Z. anal. Chem.*, 1921, 60, 239). These chemists found that many other aldehydes and ketones (and ethyl alcohol) serve the same purpose as acetone, but they recorded at the same time some exceptions so far as the nitro-bodies were concerned. Thus Bitto found that trinitroresorcinol does not give any coloration with acetone and alkali, whilst trinitroxylene gives a green colour with alkali alone. Reitzenstein and Stamm noticed that *p*-dinitrobenzene and dinitro-resorcinol do not give Janovsky's reaction, whilst some mononitro-compounds give colorations which are difficult to distinguish from those given by polynitro-compounds (e.g. *m*-nitrophenol and trinitro-phenol give identical colorations). Further instances of the failure of Janovsky's reaction which I have observed include 2:4-dinitromesitylene, trinitromesitylene, 3:5-dinitro-*o*-cresol, 2:4:6-trinitro-*m*-cresol and 3:5-dinitro-1:2:4-xylenol. It is thus evident that Janovsky's reaction is hardly suitable for even the diagnosis of aromatic polynitro-compounds.

It was thought that if the nitro-group could be dislocated as nitrous acid a method of wider applicability than those mentioned would be available, since nitrous acid can be detected easily by Griess-Ilosvay's reagent (*Bull. Soc. Chim.*, 1899, [iii], 2, 388), which is said to have a sensitiveness of 1 in 1,000,000,000. Accordingly a large number of nitro-compounds were treated with various hydrolytic agents with a view to realise a decomposition of the following type:



After numerous experiments the following procedure was found to be the most satisfactory: A small quantity (0.01–0.05 grm.) of the substance is boiled in a clean Pyrex test tube with 1 c.c. of a concentrated solution of pure potassium hydroxide (10 grms. in 6 c.c. of water) over a Bunsen flame for a period not exceeding two minutes. The colour of the solution deepens during the boiling, and finally a yellow to dark brown-red product is usually obtained, profound decomposition occurring in some cases. The test tube is then cooled under the tap and, 1 c.c. of water added. A few drops of this alkaline solution are poured into a test tube and acidified with about 1 c.c. of 50 per cent. acetic acid. On acidification, the solution usually assumes a straw-yellow colour. Should this not be the case, the solution may be diluted with water until it becomes pale in colour, and it is then treated with about 0.5 c.c. of Griess-Ilosvay's reagent, prepared according to



the directions given by Lunge (*Z. angew. Chem.*, 1899, 666).<sup>\*</sup> The development of a rose-red colour indicates the presence of free nitrous acid, and, indirectly, that of a nitro-group in the substance taken. Control experiments may be performed, omitting the nitro-compound, whenever any doubt arises as to the purity of the reagents.

I have tested fifty-two nitro-compounds with the following results:

#### REACTION POSITIVE.

- |                                |   |
|--------------------------------|---|
| 1. Nitromethane.               | 18. 3:5-Dinitro- <i>o</i> -cresol.          |
| 2. Nitroethane.                | 19. 3:4-Dinitro- <i>o</i> -cresol.          |
| 3. 1:2-Dinitrobenzene.         | 20. 2:4-Dinitrostilbene.                    |
| 4. 1:3-Dinitrobenzene.         | 21. 3:5-Dinitro- <i>p</i> -toluic acid.     |
| 5. 1:4-Dinitrobenzene.         | 22. Dinitrophthalic acid.                   |
| 6. 1:2:4-Dinitrotoluene.       | 23. 2:4-Dinitro-6-bromophenol.              |
| 7. 1:2:6-Dinitrotoluene.       | 24. 1:5-Dinitronaphthalene.                 |
| 8. 1:2:4-Chlorodinitrobenzene. | 25. 1:8-Dinitronaphthalene                  |
| 9. 1:2:4-Bromodinitrobenzene.  | 26. 1:5-Dinitro-2-methylantraquinone.       |
| 10. 1:2:4-Dinitrophenol.       | 27. Dinitro-2-methoxy-6-methylantraquinone. |
| 11. 1:2:4-Dinitroaniline.      | 28. 1:3:5-Trinitrobenzene.                  |
| 12. 1:2:4-Dinitrobenzoic acid. | 29. 2:4:6-Trinitrophenol.                   |
| 13. 1:3:5-Dinitrobenzoic acid. | 30. Trinitromesitylene.                     |
| 14. 1:2:4:6-Dinitroxylene.     | 31. 2:4:6-Trinitrotoluene.                  |
| 15. 1:2:3:4-Dinitroxylene.     | 32. Trinitroresorcinol.                     |
| 16. 2:4-Dinitromesitylene.     | 33. 2:4:6-Trinitro- <i>m</i> -cresol.       |
| 17. 3:5-Dinitro-1:2:4-xylenol. | 34. $\alpha$ -Trinitronaphthalene.          |

#### REACTION NEGATIVE.

- |                            |                                   |
|----------------------------|-----------------------------------|
| 1. Nitrobenzene.           | 10. <i>o</i> -Nitroaniline.       |
| 2. <i>o</i> -Nitrotoluene. | 11. <i>p</i> -Chloronitrobenzene. |
| 3. <i>m</i> -Nitrotoluene. | 12. <i>o</i> -Chloronitrobenzene. |
| 4. <i>p</i> -Nitrotoluene. | 13. <i>p</i> -Nitroacetanilide.   |
| 5. <i>o</i> -Nitrophenol.  | 14. <i>m</i> -Nitrobenzaldehyde.  |
| 6. <i>m</i> -Nitrophenol.  | 15. <i>m</i> -Nitrobenzoic acid.  |
| 7. <i>p</i> -Nitrophenol.  | 16. <i>p</i> -Nitrobenzoic acid.  |
| 8. <i>p</i> -Nitroanisole. | 17. $\alpha$ -Nitronaphthalene.   |
| 9. <i>p</i> -Nitroaniline. | 18. 2:2-Dinitrostilbene.†         |

It will be seen from the above tables that, with the exception of aromatic mononitro-compounds, all nitro-compounds decompose under the experimental conditions, yielding nitrous acid. It was further observed that aliphatic nitro-compounds are hydrolysed to nitrous acid, even when shaken with cold dilute alkali for a few seconds. Amyl nitrite, being an ester of nitrous acid, is also readily hydrolysed by cold dilute alkali. Consequently, it is not possible to distinguish

<sup>\*</sup> The reagent is prepared by dissolving 0.5 gm. of sulphanilic acid in 150 c.c. of 2 *N* acetic acid and mixing the solution with a colourless solution of 0.1 gm. of pure  $\alpha$ -naphthylamine in 20 c.c. boiling water and 150 c.c. 2 *N* acetic acid.

† Although its name does not imply it, this substance is really a mononitro-compound of benzene, in the sense that each of the two benzene rings carries a nitro-group and they are separated by an ethylenic linkage.

between a nitrite and an aliphatic nitro-compound by their action upon alkali.\* These may, however, be readily distinguished from aromatic polynitro-compounds, which are not so easily affected by cold alkali. It is thus possible to distinguish between the following types of nitro-bodies:

- I. *Aliphatic nitro-compounds and nitrites*: decomposed by cold dilute alkali.
- II. *Aromatic mononitro-compounds*: not affected by hot alkali.
- III. *Aromatic polynitro-compounds*: decomposed by hot alkali.

Instances of the decomposition of di- and poly-nitro-compounds into nitrous acid under the influence of alkali have been known for a long time, prominent workers in this field being Lobry de Bruyn, Holleman, Blanksma, Kenner and Jackson (for a complete bibliography dealing with nitro-derivatives of benzene, see Clark and Carter, *Trans. Roy. Soc. Canada*, 1927, [iii], 21, 322). Clark and Carter (*loc. cit.*) made an extensive quantitative study of the replaceability of nitro-groups in the benzene series by OH-groups by means of dilute alkali at 100° C. Their results, together with those of previous investigators, show that a nitro-group in the ortho- or para-position to another nitro-group is always displaced, as demanded by Fry's polarity hypothesis. They also find that, as a rule, the nitro-group in *m*-dinitro-compounds is not displaced. I have found, however, that under the experimental conditions mentioned before a nitro-group may be displaced (partly at least) from *m*-dinitro-compounds. Consequently, this method is applicable to *all* polynitro-derivatives, irrespective of the orientation of the nitro-groups in the molecule.

I have submitted *p*-nitrosophenol and *p*-nitrosodiphenylamine to the above-described process of hydrolysis, and in neither instance did I find any evidence of the formation of nitrous acid. Hence, nitroso derivatives of phenols and amines do not give the reaction.

I wish to express my sincere thanks to Sir P. C. Rây for his interest in this investigation.

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\* Alkyl nitrites may be differentiated from aliphatic nitro-compounds by Liebermann's reaction, the former giving a positive result (see Houben, *Methoden der Organischen Chemie*, 2nd Ed., Vol. IV, p. 100), or by Konowalow's test, which is negative in the case of a nitrite.

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## Determination of the Carbonyl and Aldehyde Content of Organic Compounds: Estimation of Phenylhydrazine.

By L. MARKS, M.Sc., A.I.C., AND R. S. MORRELL, M.A., Ph.D., F.I.C.

(Read at the Meeting, December 3rd, 1930).

THE object of this investigation was to discover a reliable method for the accurate determination of the carbonyl,  $>\text{CO}$ , content of certain oily and gummy substances of unknown constitution which had been isolated during the oxidation of  $\beta$ -elaeostearin from tung oil (Morrell and Marks, *J. Oil and Colour Chem. Assoc.*, 1927, 10, 186; 1929, 12, 183). Parallel investigations into the determination of the peroxide-oxygen (ANALYST, 1929, 54, 503) and of the hydroxyl contents (ANALYST, 1931, 429) of substances of this class have already been published.

Most of the methods for the determination of aldehydes and ketones can be applied only to certain substances, but the three processes described below, all of which depend on the use of phenylhydrazine, appeared to be capable of general application, and were, therefore, carefully examined. (The iodimetric method of Ardagh and Williams (*J. Amer. Chem. Soc.*, 1925, 47, 2983) is obviously unsuitable for unsaturated compounds of this type.)

1. MACLEAN'S METHOD.—I. Smedley MacLean (*J. Biol. Chem.*, 1913, 7, 611) obtained satisfactory results for pyruvic acid by treating a weighed quantity with excess of phenylhydrazine. The amount of this left over is then determined by adding excess of Fehling's solution; the precipitated cuprous oxide is dissolved in an acid solution of ferric sulphate, and the ferrous sulphate produced is titrated with potassium permanganate solution. A blank phenylhydrazine test, without the ketonic substance, is made at the same time.

A series of experiments with each of the following substances has been carried out: (a) Pyruvic acid, (b) salicylaldehyde, (c) benzaldehyde, (d) aceto-acetic ether. In each series the relative quantity of phenylhydrazine employed was successively reduced so as to determine the effect of the mass action of this reagent. The practical details given by MacLean were followed, with the following exceptions:

(i) Since an acid solution of ferric sulphate oxidises cuprous oxide in the cold, the mixture was not heated.

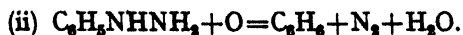
(ii) Phenylhydrazine hydrochloride, mixed with its own weight of sodium acetate, was used instead of the free base. The phenylhydrazine hydrochloride was that supplied by the B.D.H. as "specially pure for osazone tests."

(iii) The end-point in the permanganate titration was obtained with potassium ferricyanide as external indicator. This was necessary because of the difficulty, after having filtered off the cuprous oxide, of removing all traces of tartrate, the presence of which delays the end-point considerably. (See also Jones and Carpenter, *J. Chem. Soc.*, 1903, 83, 1399.)

MacLean showed that the reaction between phenylhydrazine and Fehling's solution reaches an equilibrium value in approximately half-an-hour at ordinary temperatures, after which no further oxidation takes place. We have confirmed this observation. The reaction involved approximates to the following equation:



but it varies to some extent with the conditions, the most important of which is the temperature. The following reactions may also take place:



The weight of cuprous oxide precipitated is also influenced by oxidation of the phenylhydrazine through contact with air; hence the permanganate equivalent of the phenylhydrazine must be independently determined. MacLean found that 1 c.c. of 0.1 *N* potassium permanganate solution was equivalent to 0.00295 gm. of phenylhydrazine. In our experiments the equivalent varied between 0.00330 and 0.00357 gm. The theoretical figure required by the equation (i) above is 0.00360 (*cf.* Foster, *Proc. Camb. Phil. Soc.*, 1907, 14, 90).

We have carried out two other series of preliminary experiments, which showed that the weight of cuprous oxide precipitated (i) does not vary with the amount of Fehling's solution added, provided excess of that reagent is maintained; (ii) has a slight tendency to decrease as the dilution of the reacting mixture is increased.

The following results were obtained with the substances mentioned:

(a) *Pyruvic Acid (freshly distilled).*

Expt. No.	Wt. of acid. Grm.	Phenyl- hydrazine solution (approx. 3 per cent.). c.c.	Molecular proportions pyruvic acid: phenylhydrazine hydrochloride.	Carbonyl >CO found. Per Cent.	Percentage of theoretical figure.
1	0.055	10 (0.3358 gm.)	1:5.0	22.4	70.2
2	0.056	"	1:4.9	24.0	75.2
3	0.123	"	1:2.2	26.8	84.0
4	0.183	"	1:1.5	28.2	88.4

Theoretical figure = 31.9 per cent. The purity of the sample used was not otherwise determined.

(b) *Salicylaldehyde (freshly distilled).*

Expt. No.	Wt. of salicylaldehyde. Grm.	Phenylhydrazine solution (approx. 3 per cent.). c.c.	Molecular proportions salicylaldehyde: phenylhydrazine hydrochloride.	Carbonyl >CO found. Per Cent.	Percentage of theoretical figure.
1	0.050	10 (0.3358 grm.)	1:7.6	6.2	27.1
2	0.106	"	1:3.6	17.5	76.4
3	0.208	"	1:1.8	22.7	99.1
4	0.279	"	1:1.4	23.1	100

Theoretical figure = 22.9 per cent. The purity of the sample was not otherwise determined.

(c) *Benzaldehyde.*

Expt. No.	Wt. of benzaldehyde. Grm.	Phenylhydrazine solution (approx. 3 per cent.). c.c.	Molecular proportions benzaldehyde: phenylhydrazine hydrochloride.	Carbonyl >CO found. Per Cent.
1	0.057	10 (0.3358 grm.)	1:5.8	24.5
2	0.118	"	1:2.8	24.9
3	0.196	"	1:1.7	26.6

Theoretical figure (for sample 93.5 per cent. pure) = 24.7 per cent. The sample was found by the sodium sulphite method to have a purity of 93.5 per cent.

(d) *Ethyl acetoacetate.*

Expt. No.	Wt. of acetoacetic ether. Grm.	Phenylhydrazine solution (approx. 3 per cent.). c.c.	Molecular proportions ethyl acetoacetate phenylhydrazine hydrochloride.	Carbonyl >CO found. Per Cent.
1	0.055	10 (0.3358 grm.)	1:7.3	17.3
2	0.097	"	1:4.2	18.7
3	0.216	"	1:2.4	17.6

Theoretical figure for one CO group = 21.5 per cent. The purity of the sample was not otherwise determined.

The data given permit of the following conclusions:

(1) The method is reliable only for those cases in which the hydrazone formed is not hydrolysed by water, *e.g.* for pyruvic acid and benzaldehyde. Even in these cases, however, slight hydrolysis of the hydrazone takes place, which accounts for the low results. Where the hydrazone left in solution undergoes appreciable hydrolysis (*e.g.* acetone and ethyl acetoacetate) the method fails (see also Bodforss, *Z. physik. Chem.*, 1924, 109, 223).

(2) Considerable excess of phenylhydrazine should be avoided; the best ratio is approximately  $1\frac{1}{2}$  molecular proportions of phenylhydrazine to 1 molecular proportion of the carbonyl compound.

Additional series of experiments were carried out with the following ketonic compounds: (e) Acetone, (f) laevulinic acid, (g) methyl ester of oxidised  $\beta$ -elaeostearic acid,  $C_{18}H_{30}O_6(CH_3)$  (Morrell and Marks, *loc. cit.*). With each of these substances the first conclusion (*supra*) was confirmed. The effect of variation of the molecular proportions and of the concentration was particularly pronounced with the last-mentioned compound; this is shown by the following figures:

Expt. No.	Number of molecular proportions of phenylhydrazine to one of the compound $C_{18}H_{30}O_6(CH_3)$ .	Number of molecular proportions of phenylhydrazine which reacted.	Solution.
1	30	nil	dilute
2	53	7.2	"
3	166	12.8	"
4	159	62.2	concentrated

It is possible, however, that in this case slight oxidation of the phenylhydrazine, as well as hydrazone formation, may have been taking place.

An example of an estimation of salicylaldehyde showing the calculation follows:

*Example :*

Salicylaldehyde : 1 molecular weight = 122.

Phenylhydrazine hydrochloride :  $1\frac{1}{2}$  molecular weight = 217 (approx.).

A weighed quantity (0.208 grm.) of salicylaldehyde was dissolved in about 80 c.c. of distilled water; 10 c.c. of a solution (in 50 per cent. acetic acid) of phenylhydrazine hydrochloride, containing 4.477 grms. (together with an equal weight of sodium acetate) in 100 c.c., were added; the mixture was made up to 100 c.c. and filtered, leaving, approximately, a third of the hydrazine (*i.e.*  $\frac{1}{3}$  molecular proportion) in the filtrate, 25 c.c. of which were withdrawn for treatment with Fehling's solution

Titration with  $N/10$  potassium permanganate solution = 11.0 c.c.

Therefore phenylhydrazine excess = 44.0 c.c. of  $N/10$  permanganate solution.

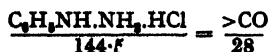
For the blank experiment 3.50 c.c. of the phenylhydrazine solution (*i.e.* about one-third of the quantity used in the previous experiment) was made up to 100 c.c., and 25 c.c. again withdrawn for the determination of the permanganate equivalent.  $N/10$  potassium permanganate titration = 8.40 c.c.

Therefore,  $N/10$  permanganate titration for 10 c.c. of the phenylhydrazine solution

$$= \frac{100}{25} \times \frac{10}{3.5} \times 8.40 = 96.0 \text{ c.c.}$$

Hence, 1 c.c. of  $N/10$  permanganate =  $\frac{0.4477}{96}$  grms. of phenylhydrazine hydrochloride.

The phenylhydrazine hydrochloride used after treatment of 10 c.c. with 0.208 grm. of salicylaldehyde =  $96.0 - 44.0 = 52.0$  c.c. of  $N/10$  permanganate



Therefore,  $>CO$  content of salicylaldehyde =  $\frac{52.0}{1} \times \frac{0.4477}{96} \times \frac{28}{144.5} \times \frac{100}{0.208} = 22.6$  per cent.

It was thought that the above-described method might be rendered more reliable by the substitution of *p*-nitrophenylhydrazine for the phenylhydrazine, since the nitro-derivatives of the hydrazones are generally less soluble in, and more

stable towards, water. Trials with this substance, however, showed that the subsequent reduction of the Fehling's solution with excess of the nitro-derivative cannot be depended upon to act quantitatively, because metallic copper is precipitated together with cuprous oxide. The introduction of an acid nitro-group in the *para* position obviously renders the compound more sensitive to oxidation, at any rate in an alkaline medium. The final conclusion with regard to the MacLean process is that, in the case of a substance of unknown composition, and in the absence of information concerning the behaviour of the hydrazone formed from it, the method is not safe.

2. WATSON SMITH'S METHOD.—This method is a modification of Strache's method, and is fully described in the reference cited (Smith, *Chem. News*, 1906, 93, 83), and in most reference works on organic analysis. [Thorpe and Whiteley, *Organic Chemical Analysis* (London), 1925, p. 182; Kingscott and Knight, *Quantitative Organic Analysis* (London), 1914, p. 121; Barnett and Thorne, *Organic Analysis* (London), 1921, p. 125.] It resembles the method just described in that the excess of phenylhydrazine left after formation of the hydrazone is treated with Fehling's solution, but instead of the cuprous oxide thereby precipitated being collected, the nitrogen evolved is collected and measured. The errors involved in the reaction between the phenylhydrazine and the Fehling's solution, with formation of cuprous oxide, are thereby avoided, but this advantage is outweighed by the very elaborate apparatus which is now necessary, and the consequent considerable errors involved. The apparatus for this process was set up independently by each of us, working in separate laboratories. A large number of determinations were carried out on substances of known carbonyl,  $>\text{CO}$ , content, but the results, independently obtained, were unsatisfactory. Figures for the same compound often showed variations of 10 per cent., or even more (in some cases too low and in others too high), and it became obvious that the data obtained for a substance of unknown composition would not permit of a definite answer as to the number of  $>\text{CO}$  groups present. This experience of the Watson Smith method is confirmed by Ardagh and Williams (*loc. cit.*), who describe it as being "tedious and cumbersome," and whose best results for acetone showed an error of 5 per cent., as well as by Ellis (*J. Chem. Soc.*, 1927, 130, 848), according to whom the process is "unnecessarily tedious and often untrustworthy." It would appear desirable, therefore, that, in future works on organic analysis, attention should be drawn to the lack of accuracy associated with this method.

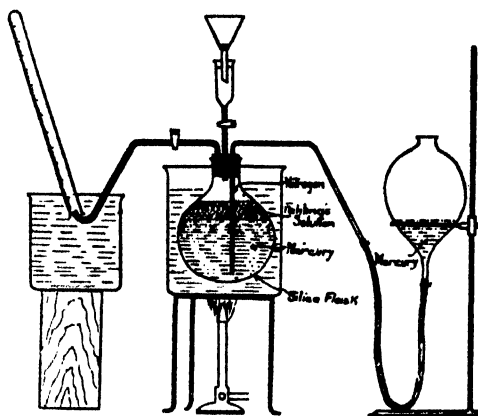
Some of the figures obtained are as follows:

Substance.	>CO found			>CO
	Per Cent.			calculated. Per Cent.
Pyruvic acid .. .. .	29.3;	28.4;	35.4	31.9
Salicylaldehyde .. .. .	26.6;	21.0;	27.2	22.9
<i>p</i> -Nitro-benzaldehyde .. .. .	23.2;	22.5		18.5
Methyl ester of oxidised $\beta$ -elaeostearic acid, $\text{C}_{18}\text{H}_{30}\text{O}_6$ ( $\text{CH}_3$ ) .. .. .	11.2;	15.3		7.8 or 15.6*

\* Either one or two CO groups may have been present in this substance.

3. **ELLIS'S METHOD.**—Some of the possible causes of the discordant results obtained by the last-described method are as follows:—(a) Incomplete formation of the hydrazone, (b) hydrolysis of the hydrazone, (c) the hydrazone is itself attacked by the boiling Fehling's solution, (d) some of the evolved nitrogen remains dissolved in the reacting mixture or in the benzene-absorbent solution, or in the potash solution below the eudiometer, (e) atmospheric oxidation of the phenylhydrazine.

Of these, (a), (b) and (c) produce low results; (e) leads to high results; the effect of (d) will be to produce a low or a high result according to whether more or less nitrogen is retained in the actual estimation than in the blank experiment. Ellis (*loc. cit.*) has modified the details for the collection of the nitrogen so as to ensure that no loss takes place. The nitrogen is completely evolved from the



boiling reaction mixture by reducing the pressure in the containing flask, which for this purpose is connected with a mercury reservoir (see diagram). In addition, the benzene vapour, which is simultaneously evolved, is not absorbed, but its presence is allowed for in the subsequent calculation. Although the apparatus is thus modified considerably, it must be specially made for the purpose, and involves (for safety) also a silica flask. Some of the results obtained by us with it are set out below. In each case the substance was treated with approximately  $1\frac{1}{2}$  molecular proportions of phenylhydrazine.

Compound.	>CO found.	>CO
	Per Cent.	calculated. Per Cent.
Phenylhydrazine hydrochloride A.R. ..	98.2; * 101*	100*
Salicylaldehyde redistilled .. ..	22.0; 23.1	22.9
Acetone A.R. .. ..	48.6; 47.3	48.2
Pyruvic acid .. ..	29.5	31.9
Oxidised $\beta$ -elaeostearin ( $C_{18}H_{29}O_5$ ) <sub>3</sub> C <sub>3</sub> H <sub>5</sub>	8.3; 9.4	8.3

\* Percentage purity.

It should be mentioned that in our experiments, (a) any precipitated hydrazone was removed by filtering through glass wool or asbestos fibre in a small



porcelain funnel fitted into the cup of the apparatus; (b) the special pipette described by Ellis was not used; the reacting solutions were mixed in a very small beaker, and the freshly boiled distilled water, alcohol or glacial acetic acid that was used for washing the hydrazone was run out of a burette, the same volume being used in the blank; (c) it was found that the apparatus as sketched has the advantage over that of Ellis in that the phenylhydrazine solution can now be decomposed and the nitrogen collected *in portions*, if necessary; the use of a two-way stopcock necessitates, for accurate results, that *all* the phenylhydrazine and the washings are run into the Fehling's solution before any nitrogen is driven over into the eudiometer, because of the small volume of liquid which is retained in the tube between the two-way stopcock and the flask; (d) the nitrogen collected was saturated with benzene vapour by introducing a drop into the eudiometer, and the partial pressure of the benzene was then allowed for by reading the graph constructed from the data given by Woringer (*Z. physik. Chem.*, 1900, **34**, 257); (e) glacial acetic acid, or 50 per cent. aqueous acetic acid solution, was employed as a solvent, when the ketonic compound was insoluble in both water and alcohol; care was taken in these cases to maintain the Fehling's solution in the silica flask alkaline by addition of more potassium hydroxide.

It is advisable to filter off the hydrazone, because it is liable to undergo hydrolysis, with the result that the volume of nitrogen evolved is high and the figure for ketonic content is low. This is liable to take place, for instance, in the case of acetone, the hydrazone of which, being soluble, cannot be removed. At the outset there is a vigorous evolution of nitrogen (from the excess of phenylhydrazine), followed by a further slow evolution; the initial rapid evolution is always greater when the reaction mixture is allowed to stand for a few hours before being introduced into the Fehling's solution.

Ellis's process was finally adopted as the most rapid and satisfactory for the determination of the ketonic content of a large number of oily complex substances of unknown constitution (Morrell and Marks, *J. Soc. Chem. Ind.*, 1931, **50**, 277).

It will be observed that the blank experiment in the above estimations is, in effect, the determination of the amount of phenylhydrazine solution used. It is obvious that the procedure can be applied to the estimation of the purity of a sample of phenylhydrazine. The results vary by  $\pm 2$  per cent.

**SUMMARY.**—The use of phenylhydrazine and Fehling's solution in three methods (due to MacLean, Watson Smith, and Ellis) for the determination of the carbonyl,  $>\text{CO}$ , content in aldehydes and ketones has been investigated. The MacLean method is not safe in the case of a substance of unknown composition, and in the absence of information concerning the behaviour of the hydrazone formed from it. The Watson Smith method is unreliable. Ellis's modification of the Watson Smith method gives satisfactory results, and it can also be used for the estimation of phenylhydrazine.

# The Reichert, Polenske and Kirschner Values of Rancid Butters and Margarines.

By G. D. ELSDON, B.Sc., F.I.C., R. J. TAYLOR AND P. SMITH.

(Read at the Meeting of the North of England Section, December 6, 1930.)

At one of the meetings of the North of England Section of the Society, during the discussion following a paper by two of us, an enquiry was made regarding the effect, if any, of keeping (and, therefore, presumably, the action of rancidity) on the Reichert, Polenske and Kirschner value of fats. Since then we have had an opportunity of investigating these points rather more fully than had been the case up to that time, and we now present the results obtained.

It is, of course, well known that when oils become rancid there is a very considerable increase in the volatile acids present. (Elsdon and Hawley, *Year Book of Pharmacy*, 1913, p. 575.) Some years ago one of us extracted from a rancid sample of cotton-seed oil a considerable quantity of fatty acid which had a valeric odour, but this was not further examined. Apart from such experiences as this, no work appears to have been published showing what increases may be expected in the case of ordinary fats. Certain old samples of butter have been examined (Radclyffe and Maddocks, *J. Soc. Chem. Ind.*, 1907, **26**, 3), and D. Crispo (*ANALYST*, 1911, **36**, 64) found that some samples of butter increased in their Reichert value with age, and some lost, but that, on the whole, the losses were greater than the gains.

For our present purpose, certain mixtures of coconut oil, palm-kernel oil, margarine and butter fats, which were prepared for special reasons in 1925 and 1926, were used, as these had been placed on one side in open beakers of about 250 c.c. capacity in a dark cupboard at a medium temperature, and with free access to air. They had been standing for some fourteen months. When the Reichert value was being determined, it was observed that the fat turned dark brown when saponified with sodium hydroxide and that the fatty acids, on liberation, were more liquid than they were in the original samples. The figures obtained with these samples, both when freshly prepared and after standing for fourteen months under the conditions mentioned above, are given in the following tables:

TABLE I.  
PALM-KERNEL OIL AND OLEO-MARGARINE.

Composition.	Reichert value.		Polenske value.		Kirschner value.	
	Dec. 1925.	Feb. 1927.	Dec. 1925.	Feb. 1927.	Dec. 1925.	Feb. 1927.
20 per cent. Palm-kernel oil	1.8	2.1	1.7	1.8	0.5	0.6
40   "       "       "	2.6	4.0	3.2	3.7	0.6	1.2
80   "       "       "	4.3	5.5	7.4	7.4	0.9	1.5
*20   "       "       "	1.8	3.7	1.5	2.1	0.5	1.4

\* Prepared from different ingredients, February, 1925, and determinations made then and after two years.

TABLE II.

## COCONUT OIL, PALM-KERNEL OIL AND OLEO-MARGARINE.

Composition.	Reichert value.		Polenske value.		Kirschner value.	
	Dec. 1925.	Feb. 1927.	Dec. 1925.	Feb. 1927.	Dec. 1925.	Feb. 1927.
20 per cent. Palm-kernel oil	4.2	5.7	4.4	4.4	0.6	1.8
20    "   Coconut oil						
30    "   Palm-kernel oil	5.2	6.7	7.2	7.2	0.9	2.0
30    "   Coconut oil						
40    "   Palm-kernel oil	6.2	7.0	9.5	10.3	1.2	2.1
40    "   Coconut oil						
50    "   Palm-kernel oil	6.7	7.4	13.0	13.5	1.4	2.2
50    "   Coconut oil						

TABLE III.

## PALM-KERNEL OIL, BUTTER-FAT AND OLEO-MARGARINE.

Composition.	Reichert value.		Polenske value.		Kirschner value.	
	Feb. 1925.	Feb. 1927.	Feb. 1925.	Feb. 1927.	Feb. 1925.	Feb. 1927.
60 per cent. Palm-kernel oil	6.0	8.0	6.0	6.3	3.3	4.3
10    "   Butter-fat						
40    "   Palm-kernel oil	5.2	6.7	3.5	4.1	3.0	3.6
10    "   Butter-fat						
50    "   Palm-kernel oil	5.6	8.0	4.7	5.0	3.2	4.1
10    "   Butter-fat						
20    "   Palm-kernel oil	4.4	6.6	1.8	2.4	3.0	4.5
10    "   Butter-fat						
*20    "   Palm-kernel oil	4.5	7.5	1.8	2.5	2.8	5.2
10    "   Butter-fat						
20    "   Palm-kernel oil	2.2	4.2	1.5	2.1	0.9	2.2
2    "   Butter-fat						

\* Mixture prepared from different ingredients, January, 1925.

From an examination of these tables, it will be observed that the Reichert value has increased in every case, and that in some cases the increase is very marked, corresponding with a considerable increase in the apparent percentage of butter-fat present. The Polenske value has increased in some cases, but usually by a much smaller proportion than the Reichert. The Kirschner value has also increased in every case, but there is no obvious relationship between the increase in the Kirschner value and the increase in the Reichert value.

All these results suggest that when the amount of butter-fat present in the margarine mixture is determined by means of the Reichert, Polenske and Kirschner process, if this is not carried out while the margarine is quite fresh, special care should be taken in the interpretation of the results obtained.

In view of these results, samples of butter-fat were examined after various lengths of time. Five samples of butter were melted, the fat filtered, and about 35 grms. placed in glass crystallising basins. A portion of each was allowed to stand in a dark cupboard covered with paper, and another portion of each was allowed to stand uncovered on the bench. The samples were examined after nineteen weeks, with results as given in the following table:—

TABLE IV.

No. of sample.	Reichert value.			Polenske value.			Percentage of free fatty acids.*		
	Original.	in dark.	in light.	Original.	in dark.	in light.	Original.	in dark.	in light.
1	30.5	31.0	31.2	2.7	3.6	3.7	0.28	0.79	1.29
2	30.1	30.6	30.9	3.2	3.8	4.2	0.33	1.07	1.12
3	30.4	30.3	31.0	3.3	3.4	4.0	0.28	0.39	1.01
4	29.9	29.7	30.6	2.6	2.8	3.4	0.50	0.50	1.01
5	29.9	29.9	30.2	2.7	3.1	3.4	0.45	0.62	1.1
Sample of lard	0.2	—	1.6	0.3	—	0.6	0.62	—	1.58

\* Expressed as oleic acid.

From these results it would appear that the Reichert value of butter is not changed very much when exposed to the activities of light and air over a period of a considerable number of weeks, under conditions when the free fatty acids have risen to as much as 1.3 per cent. It is apparent that, as would be expected, the increase in acidity is very much more in the case of those samples which are exposed to light than in the case of those samples which are kept in the dark. The increase in the Polenske value is quite marked, particularly in those samples which are exposed to the light.

A number of samples of canned butter were very kindly placed at our disposal by Messrs. Nestlé, to whom our thanks are due. These samples had been canned for approximately twelve months at the time the determinations were made. The results obtained are set out in the following table:

TABLE V.

Mark.	Reichert value.	Polenske value.	Kirschner value.	Free fatty acids. Per Cent.
SP. 1095	29.1	2.6	24.45	1.01
SP. 1098	29.0	2.5	24.75	0.95
SP. 1097	29.1	2.5	24.7	0.62
SP. 1102	27.35	2.9	23.75	0.95
SP. 1096	27.6	2.6	23.65	1.41
SP. 1099	27.6	2.5	23.35	2.54
SP. 1100	25.85	2.2	22.3	1.01
SP. 1101	26.8	2.3	22.75	0.90
SP. 1103	30.35	3.1	25.9	1.80
SP. 1104	24.6	1.6	21.35	6.20

Beyond the considerable increase in the percentage of free fatty acids there is little to distinguish these results from those obtained with fresh samples.

A quantity of butter-fat obtained by mixing eleven samples of butter, received under the Food and Drugs Act, melting and clarifying, was allowed to stand for nineteen months uncovered in a dark cupboard at laboratory temperature. During this time, the Reichert value increased from 28.0 to 28.9, whilst the Polenske value remained unchanged at 2.1.

There is, as a general rule, therefore, little increase in the Reichert values of butter fats as these become rancid, or at least not sufficient to invalidate the results obtained under any likely commercial conditions.

P. Arup (ANALYST, 1929, 54, 736) has shown that during a period of nearly six years the Reichert value of a sample of butter kept in a bottle fitted with a screw cap and cork disc fell from 20.7 to 8.0, but other samples, although behaving in the same way, did not do so to the same extent.

The available evidence (*cf.* J. S. Hepburn, *7th International Congress of Applied Chemistry*, 1909, Sec. VIII C., p. 268) would suggest that during comparatively short periods of time there is a tendency for volatile acids to be produced as oils become rancid, but that under certain conditions these may be consumed or decomposed by biological agencies. The somewhat contradictory results which have been published are probably due to different types of organisms causing varying types of decomposition.

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## The Determination of Small Quantities of Phosphate by Pouget and Chouchak's Method: the Determination of Phosphorus in Small Samples of Steel.\*

BY S. G. CLARKE, PH.D., A.I.C.

THE necessity has arisen for methods for the analysis of steel which are suitable for dealing with small samples. Such a method for the determination of sulphur in steel has already been published (Clarke, ANALYST, 1931, 436), and the present paper contains the results of an investigation of a method for the determination of phosphorus in small samples of steel weighing 0.1 grm. As in the case of sulphur, a photometric method of determination was called for, owing to the minute quantities of phosphorus in question.

There are in existence two main methods for the determination of traces of phosphate, depending, respectively, upon the turbidity produced by the formation of phosphomolybdates of certain bases, in particular strychnine, and upon Denigès'

\* Communication from the Research Department, Woolwich.

discovery that, under certain conditions of acidity of the solution, etc., blue reduction products of molybdenum can be formed only in the presence of phosphate or arsenate. While this latter type of method is attractive on account of its great sensitiveness, it would appear from recent work (Truog and Meyer, *Ind. Eng. Chem., Anal. Ed.*, 1929, 1, 136) that there is a serious interference even by small amounts of iron. There is, therefore, little prospect of this method being applied to the determination of phosphorus in steel, owing to the difficulty in separating traces of phosphorus from iron in a simple manner.

The method to be described is based upon the work of Pouget and Chouchak (*Bull. Soc. chim.*, 1909, 5, [iv], 104; 1911, 9, 649) who, in their papers on the determination of minute amounts of phosphoric acid by means of the turbidity produced by the formation of strychnine phosphomolybdate, suggest a method for determining phosphorus in steel. While the investigation of these authors is pioneer work of the highest value, a re-investigation of the subject was found to be justified.

**THE REAGENT.**—The strychnine-molybdic acid reagent, as used in this work, is prepared as follows: Neutral sodium molybdate\* (33 grms.) is dissolved in water (150 c.c. approx.), and nitric acid (50 c.c. of 1.42 sp. gr.) added slowly, the solution being well shaken during the addition; the resulting clear solution is cooled and diluted to 250 c.c. To a portion of this stock solution (which keeps well) is added, also with vigorous shaking, one-tenth of its volume of a hot solution of strychnine sulphate, prepared by dissolving strychnine (2 grms.) in hot water to which a few c.c. of dilute (1:3) sulphuric acid have been added, and diluting to 100 c.c.† A slight precipitate appears on adding the strychnine solution; the reagent is filtered through a No. 42 Whatman paper before use. This strychnine-molybdic acid reagent is not very stable and, on being kept for a few hours, becomes opalescent and develops a distinct yellow colour; it should, therefore, be freshly prepared for each series of determinations.

Kober and Egerer (*J. Amer. Chem. Soc.*, 1915, 37, 2373) have suggested a strychnine-molybdic acid reagent for the nephelometric determination of phosphate, in which nitric acid is replaced by hydrochloric acid, claiming that it is colourless and stable. This claim was not completely verified. A specific objection exists to the use of this hydrochloric acid reagent for the photometric determination of phosphorus in presence of iron, since, whereas one decigram of iron (as ferric nitrate) in 50 c.c. of solution containing nitric acid is very nearly colourless, the addition of hydrochloric acid causes the development of a decided yellow colour which would interfere with the estimation of turbidity in the liquid. This hydrochloric acid reagent has been used by Kober (*J. Ind. Eng. Chem.*, 1918, 10, 560; Yoe, *Nephelometry*, p. 144) for the nephelometric determination of phosphorus in *iron* (presumably of high phosphorus content); this method could not be used for steel, since the larger weight of sample necessary would introduce sufficient iron into the test solution to vitiate the process.

\* See note at end of paper.

† This solution deposits strychnine sulphate on cooling, and it therefore requires to be heated on a steam bath before use.

Steps have been taken to ascertain the most suitable conditions for the formation of a satisfactory turbidity of strychnine phosphomolybdate upon which quantitative measurements could be made. In a solution of very low acidity the addition of the reagent causes the formation of a turbidity in the absence of phosphate, and large amounts of acid seriously retard the formation of visible strychnine phosphomolybdate. The presence of 10 c.c. of nitric acid (sp. gr. 1.2) in a final volume of 50 c.c. appears to be the optimum concentration, and, on the addition of 5 c.c. of the reagent, which tests had shown to be a suitable quantity, a turbidity quickly appears in the solution (containing a few hundredths of a milligram of phosphorus as orthophosphate) and reaches a steady amount in about ten minutes, thereafter remaining practically constant during the next half-hour.

The density of the turbidity has been found, contrary to the statements of Pouget and Chouchak, not to be directly proportional to the amount of phosphorus added. In order to determine phosphorus by comparison of the turbidity produced with that produced under the same conditions by a known amount of phosphate, it is necessary to apply a correction.

The correction to be applied has been determined, both in the presence and the absence of iron, by preparing series of turbidities with known amounts of phosphorus (added as a standard solution of potassium dihydrogen phosphate) and obtaining the ratio of the opacity of each member of the series to that of a given one of the members. From the results obtained, curves were drawn from which the correction to be applied, within the range of amounts of phosphorus examined, can be read.

The photometric comparison of the turbidities of strychnine phosphomolybdate was carried out, in the present work, with the use of *transmitted* light, and not by what is known as nephelometry, which employs the reflected and diffracted light produced by a turbidity under illumination normal to the length of column. The method employed was adopted because the turbidity in question is not strictly colourless. A Klett colorimeter was used, which permits of the accurate measurement of the ratio of the lengths of the columns of two unequal turbidities when the amount of light transmitted by each is the same. The light transmitted, as viewed in the divided field of the instrument, is clear yellow. The cups for holding the turbid liquid were those supplied for ordinary colorimetric work, *i.e.* made of black glass with clear fused-on bottoms; the black glass cups are essential to screen the turbid liquid from incidental light.

**METHOD FOR PURE PHOSPHATE SOLUTION.**—The method, which can be used for the determination of phosphorus in a pure orthophosphate solution containing from 0.01 to 0.06 mgrms. of phosphorus, is as follows:

Into two glass cylinders graduated at 50 c.c. (Nessler glasses are suitable) are run 10 c.c. of nitric acid (sp. gr. 1.2) from a pipette. The neutral phosphate solution is placed in one of the cylinders, and 3 c.c. of a standard phosphate solution (0.0439 gm.  $\text{KH}_2\text{PO}_4$  per litre; 1 c.c. = 0.01 mgrm. P.) is run into the other. Water

is added to both up to approximately 40 to 45 c.c., followed by exactly 5 c.c. of the strychnine and molybdcic acid reagent. The solutions are diluted to 50 c.c. and stirred by a glass plunger (say six strokes up and down). After being kept for 10 minutes, sufficient of the solutions (about 10 c.c.) are poured into the cups of the colorimeter, and when the divided field of view in the instrument has been made uniform by raising or lowering either of the cups (the standard being preferably set at a definite depth) the ratio of the depths of the solutions is measured. From this ratio the apparent amount of phosphorus is obtained. The true amount is ascertained by reference to the curve (Figure 1).

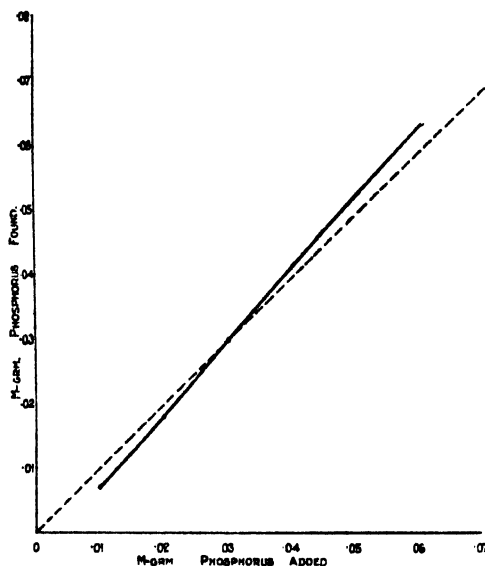


Fig. 1.\*

Table I shows the apparent amounts found by comparing turbidities from known amounts of phosphorus with a 0.030 mgrm. standard.

TABLE I.

Phosphorus added. Mgram.	Depth ratio. (0.030 mgrm. standard.)	Apparent phosphorus found. Mgram.
0.010	10/40.4	0.007
0.020	25/41.3, 20/33.4	0.018
0.030	Used as standard	0.030
0.040	30/21.6, 20/14.7	0.0415
0.050	20/11.3	0.053
0.060	30/14.0, 20/9.4	0.064
0.070	30/11.2, 20/7.8	0.080

\* For use in this process, this curve must be drawn on a large scale on squared paper, using the data in Table I.



**EFFECT OF SILICON AND ARSENIC.**—The observation of Pouget and Chouchak (*loc. cit.*), that silicon does not interfere, has been confirmed, a correct result for phosphorus having been obtained in presence of several mgrms. of this element (added as sodium silicate). Their statement that arsenic interferes has been verified. Whilst, on the one hand, it has been found that comparatively large amounts of arsenic (added as a neutralised solution of sodium arsenate) give no turbidity when treated by the method described for phosphorus, *e.g.* with 0.5 mgrm. of arsenic alone, no turbidity was produced within one hour; on the other hand, a solution containing 0.03 mgrm. of phosphorus and 0.1 mgrm. of arsenic gave a turbidity in ten minutes, corresponding with 0.056 mgrm. of phosphorus. In Kober's method for determining phosphorus in iron, the effect of arsenic is ignored.

**PHOSPHORUS IN STEEL.**—Iron in the ferric condition seems to exercise no very marked effect on the development of the strychnine phosphomolybdate turbidity; but it has been found that, in order to obtain a quantitative measure of this turbidity in the presence of iron, it is necessary that the standard turbidity should be produced in the presence of the same amount of iron. The principal requirements of a method for the determination of phosphorus in the solution of a steel in acid are:—(i) That the whole of the phosphorus should be converted into the form (orthophosphate) in which it will react with the reagent; (ii) that arsenic must be eliminated; (iii) that the final solution in which the turbidity is to be produced must be of the correct degree of acidity and should contain the iron in a form which shows the least possible neutral colour. Halogen acids must be absent.

The following method provides for these requirements:

**METHOD.**—The sample of steel and the standard (see note below) are treated in exactly the same way. The directions given are to be taken as applying to both.

One-tenth grm. is dissolved in 5 c.c. of dilute nitric acid (sp. gr. 1.2) by heating in a 400 c.c. squat beaker. Nitrous fumes having been dispelled, three drops of a saturated solution of potassium permanganate are added, the liquid is evaporated to dryness, and the residue is heated for 5 to 10 minutes. The residue is dissolved by warming with 5 c.c. of concentrated hydrochloric acid. One c.c. of hydrazine hydrochloride solution (20 per cent.) is added, the liquid is evaporated to dryness, and the brown residue is heated until it changes to yellowish-white and a slight evolution of white fumes occurs. To the residue are added 5 c.c. of concentrated hydrochloric acid, and the liquid is again evaporated to dryness. (This second evaporation is suggested as being necessary to remove all traces of arsenic.)

The residue is covered with 5 c.c. of concentrated hydrochloric acid and the beaker heated; a similar volume of concentrated nitric acid is added, and the heating continued until the vigorous effervescence has subsided.

About 30 c.c. of hot water are now added, and the liquid rendered slightly alkaline with ammonia. The liquid is boiled for a few moments and filtered through a 12.5 cm. Whatman paper (No. 41). The beaker is washed out twice with dilute potassium nitrate solution (5 per cent.), and the liquid poured on the filter, which is finally washed with two successive quantities of the same solution.

The filter paper is opened out in the funnel, and the ferric hydroxide washed back into the original beaker, *using not more than 25 c.c. of water* (a brown stain generally remains on the paper, but this is neglected). Ten c.c. of dilute nitric acid (sp. gr. 1.2) are added from a pipette to the contents of the beaker, a very small granule of potassium *nitrite* is also added, the liquid is heated (not boiled) until a clear solution is obtained, and is finally cooled in a bath of running water.

The liquid is filtered, to remove shreds of filter paper, through a small wad of damped cotton wool contained in a small funnel, a small volume of water being used to rinse out the beaker and to wash the filter. The filtrate is collected in a 50 c.c. Nessler cylinder, and its volume should be *not more than 45 c.c.*

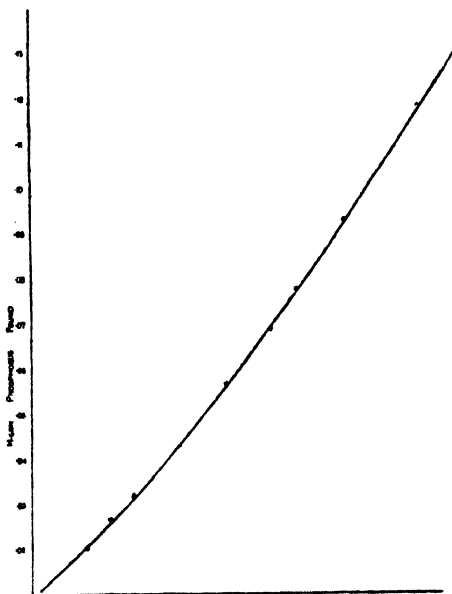


Fig. 2.\*

To the solution from the steel to be tested, and also to the standard which has been treated in the same way, are added exactly 5 c.c. of the reagent (the preparation of which is described in the earlier part of the paper), the volumes are adjusted to 50 c.c., and each solution mixed by stirring six times with a glass plunger.

After the lapse of 10 minutes, the two colorimeter cups are filled with the respective liquids, and the ratio of the opacities of the liquids determined by finding the depth of liquid which transmits the same amount of light as a definite depth of the standard liquid, *i.e.* when the divided field seen in the eye-piece of the instrument is uniform.

\* For use in the process, this curve must be drawn on a large scale on squared paper, using the data in Table II.

The apparent phosphorus content of the sample is simply

$$\frac{x}{y} \times s$$

where  $x$  represents the depth (in mm.) of the standard liquid;  $y$  the depth (in mm.) of the sample; and  $s$  the phosphorus content of the standard. The true phosphorus content of the sample is obtained by reference to the curve (Fig. 2).

**THE STANDARD.**—Two alternative standards can be used. Probably the most convenient in ordinary work would be a standard steel. Since the essential correction curve connecting the apparent phosphorus found with the true phosphorus content was worked out by using a standard containing 0.032 mgrm. of phosphorus with 0.1 grm. of iron (0.032 per cent.), a standard steel giving very nearly this value must be chosen. British Chemical Standard Steel "0.1" (0.031 per cent. P) is suitable.

In the present work 0.1 grm. of electrolytic iron was used, to which 0.030 mgrm. of phosphorus was added (as a standard solution of phosphate added to the iron in a beaker before dissolving). Analyses by independent methods showed that the electrolytic iron used contained 0.002 per cent. of phosphorus.

**THE CALIBRATION CURVE.**—The data for this curve were obtained by adding known amounts of phosphorus (as a standard solution of potassium dihydrogen phosphate) to 0.1 grm. samples of electrolytic iron, which were then submitted to the process just described. Each member of the series (except that containing 0.032 mgrm. of phosphorus) was compared in turn, the 0.032 mgrm. member being used as the standard. The results shown in Table II were obtained.

TABLE II.

Electrolytic iron. Grm.	Added.		Depth ratio. 0.032 mgrm. standard.	Found. Apparent phosphorus. Mgrm.
	Phosphorus (as std. solution). Mgrm.	Total phosphorus. Mgrm.		
0.1	0.010	0.012	10/29.3, 15/44.4	0.011
0.1	0.020	0.022	15/24.2, 20/31.6	0.020
0.1	0.025	0.027	20/24.2, 30/35.2	0.027
0.1	0.030	0.032	Used as the standard	
0.1	0.035	0.037	20/18.1, 30/27.4	0.035
0.1	0.040	0.042	20/15.0	0.043
0.1	0.050	0.052	20/11.3, 30/16.7	0.057
0.1	0.060	0.062	20/9.6, 30/13.5	0.069
0.1	0.065	0.067	30/12.3	0.078
0.1	0.075	0.077	20/6.9, 30/10.0	0.094
0.1	0.100	0.102	30/7.2	0.133

These results are plotted as a curve in Fig. 2.

Some results which have been obtained by this method of the determination of phosphorus in various standard steels are recorded in Table III.

TABLE III.

Standard used: 0.1 grm. of electrolytic iron (containing 0.002 per cent. of phosphorus + 0.030 mgrm. of phosphorus added as  $\text{KH}_2\text{PO}_4$ ).

Taken.			Found.			
Steel.	Wt. Grm.	Depth ratio.	Apparent phosphorus. Mgrm.	Corrected phosphorus (from Fig. 2).		Mean certificate result.
				Mgrm.	Per Cent.	Per Cent.
B.C.S. A <sub>2</sub>	0.1	10/49.0, 5/24.4	0.007	(0.007	0.007)	0.008
B.C.S. H <sub>1</sub>	0.1	20/30.6, 30/46.3	0.021	0.023	0.023	(0.024) <sup>1</sup>
B.C.S. O <sub>1</sub>	0.1	20/20.8, 30/31.1	0.031	0.031	0.031	0.031
B.C.S. N <sub>1</sub>	0.1	20/19.0, 30/28.7	0.033	0.033	0.033	0.036
B.C.S. S <sub>1</sub>	0.1	20/11.4, 30/17.1	0.056	0.052	0.052	0.051
B.C.S. V <sup>2</sup>	0.1	20/25.6, 30/39.2	0.025	0.026	0.026	0.024
B.C.S. P	0.1	20/5.0, 30/7.2	0.131	0.099	0.099	0.105
Bureau of Stds. B.	32 <sup>3</sup>	15/40.3	0.012	0.013	0.013	0.016

<sup>1</sup> This is the value obtained by the author with the ammonium phosphomolybdate process. The certificate result (0.027 per cent. of phosphorus) was not confirmed.

<sup>2</sup> Chromium-vanadium steel: Cr, 0.86 per cent.; V, 0.27 per cent.

<sup>3</sup> Nickel-chromium steel: Cr, 0.64 per cent.; Ni, 1.21 per cent.

NOTE ON THE PREPARATION OF SODIUM MOLYBDATE.—According to Pouget and Chouchak (*loc. cit.*) at least two substances go under the name of sodium molybdate: neutral sodium molybdate  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , and acid sodium molybdate  $\text{Na}_2\text{Mo}_7\text{O}_{24} \cdot 22\text{H}_2\text{O}$ , and it is not a matter of indifference which substance is used in the preparation of the reagent which they specify. In order to be free from uncertainty, Pouget and Chouchak gave directions for preparing their reagent from molybdic acid by dissolving this in a certain quantity of sodium carbonate solution and adding a specified amount of nitric acid. The two specimens of molybdic acid which were available to me contained combined ammonia, so that the preparation of a reliable reagent from molybdic acid appeared to be no more certain than its preparation from commercial sodium molybdate. Neutral sodium molybdate for the preparation of the reagent used in the present work was made from molybdic acid as follows:

Molybdic acid (72 grms.) is dissolved in sodium hydroxide solution (150 c.c. containing 45 grms. of NaOH) contained in a Pyrex vessel. The solution is boiled for 30 minutes to expel ammonia (if present). The solution is cooled, filtered if necessary, and the sodium molybdate precipitated by the addition of about two volumes of alcohol. It is filtered on a Buchner funnel, washed several times with alcohol and dried in an oven at 100° C.

## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### VOLUMETRIC DETERMINATION OF SULPHATE.

MODERN methods of boiler-feed water treatment require the maintenance of a definite ratio between sulphate and carbonate, the sulphate being calculated as sodium sulphate, and the alkalinity, made up of hydroxide and carbonate, as sodium carbonate.

This ratio is determined by the boiler pressure, and should be capable of being measured quickly and easily by a boiler attendant or a shift engineer.

Now, the total alkalinity is easily carried out, the two methods for such a determination suitable for boiler water being (1) the Winkler method; (2) the excess acid method. The details of these are well known and need not be described.

For the sulphate determination, the only method recommended for boiler room purposes is the turbidity method, consisting in adding barium chloride to an aliquot portion of water and measuring the turbidity of the solution against a standard.

Differences in size of the barium sulphate particles upset the calculations, and the following method appears to offer definite advantages and can be carried out with the same apparatus as that used for the alkalinity test.

The method consists in shaking a measured quantity of water with a suspension of barium carbonate, and titrating the sodium carbonate formed, with phenolphthalein as indicator.

The barium carbonate suspension can be prepared either by shaking barium carbonate in water or by mixing equivalent amounts of barium chloride and sodium carbonate dissolved in water. The supernatant liquor is tested with a drop of sodium carbonate solution to make sure that there is no soluble barium salt present, and the precipitate is filtered off on a Buchner funnel and washed. The washing need not be complete.

The precipitate is re-mixed with fresh distilled water to make a convenient suspension of the carbonate and water, and a few drops of phenolphthalein solution are added. If a purple colour develops, *N*/10 hydrochloric acid is added, drop by drop, until only the faintest colour is left.

The determination is carried out as follows:—One hundred c.c. of water are placed in a conical flask of about 300 c.c. capacity. Ten drops of phenolphthalein solution are added, and *N*/10 acid run in, drop by drop, until the liquid is colourless. The burette reading is then taken, and about 10 to 20 c.c. of barium carbonate suspension are added to the flask. A pink colour immediately develops, due to the interaction of the barium carbonate and soluble sulphate, with formation of barium sulphate and soluble carbonate. The acid is now run in slowly until the pink colour is discharged.

Care should be taken not to run the acid in too quickly, lest it should act upon the suspension of barium carbonate, but with care there is no apparent action until the soluble carbonate has been converted into bicarbonate. The number of c.c. of acid used:  $\times 96 =$  parts per million  $\text{SO}_4$ , or  $\times 142 =$  parts per million of sodium sulphate.

Whilst the method is not suggested as one to replace the gravimetric method as a final check, yet it can be carried out quickly and is sufficiently accurate to determine whether the sulphate to carbonate ratio is being maintained in the boiler. For example, a water was tested by the gravimetric and the volumetric methods and the results obtained were as follows: Gravimetric method, 1572 parts per million  $\text{SO}_4$ ; volumetric method, 1585 parts per million  $\text{SO}_4$ .

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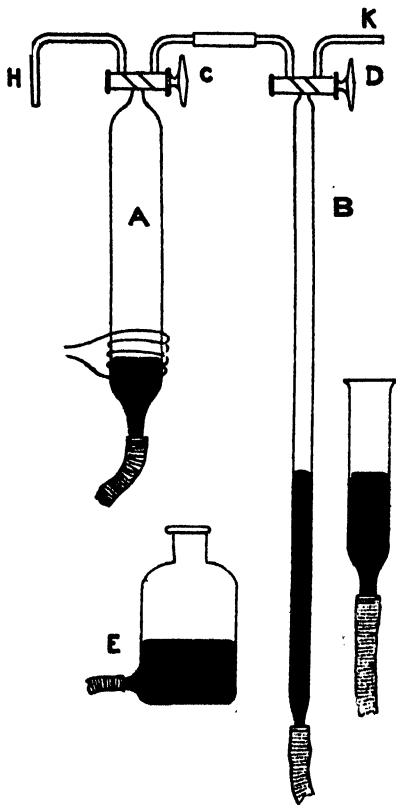
## AN APPARATUS FOR THE DETERMINATION OF SMALL QUANTITIES OF AMINO NITROGEN.

THE determination of amino nitrogen present in solution at low concentration appears to be a matter of some difficulty; but the apparatus shown in the diagram enables one part of amino nitrogen per million parts of solution to be determined.

At the outset it should be pointed out that the low pressures and small amounts of gases involved in the process render efficient lubrication of the stopcocks necessary. The paraffin and rubber lubricant supplied by British Drug Houses, Ltd., is eminently satisfactory for the purpose.

Referring to the diagram, *A* is the reaction vessel, of 100 c.c. capacity. The lower end is wrapped with several turns of resistance wire, which heats the tube sufficiently with a current of 1–2 amperes. *B* is a 10 c.c. measuring tube, graduated in hundredths of c.c.

PROCEDURE.—Stopcocks *C* and *D* are turned, so that on raising *F*, the mercury completely fills *B* and runs into *A*. *D* is then turned off, and *C* turned so that, on raising *E*, mercury fills *A* and the side tube *H*. By lowering *E*, the sample solution (as much as 50 c.c.) may be drawn through *H*, into *A*, followed by 1 c.c. of water to wash the tube. If any air has entered, it must be forced out by raising *E* so that the liquid just reaches the stopcock *C*, which is then closed. *E* is then lowered, so that a Torricellian vacuum exists above the surface of the solution. *A* is now heated (electrically), and the dissolved gases expelled. These are driven out of *A* after a few minutes, and their complete removal is shown when only a very small bubble exists between the surface of the solution and the stopcock upon levelling the mercury. After this, *C* is turned and the solution allowed just to fill *H*; *C* is then turned off and *E* lowered. Two to 4 c.c. of glacial acetic acid, contained in a small tube, are drawn in at *H* by opening *C*, care being taken that no air enters, after which 2 c.c. of 5 per cent. sodium nitrite solution, contained in another tube, are made to follow the acid, and both are drawn into *A*. *C* is then



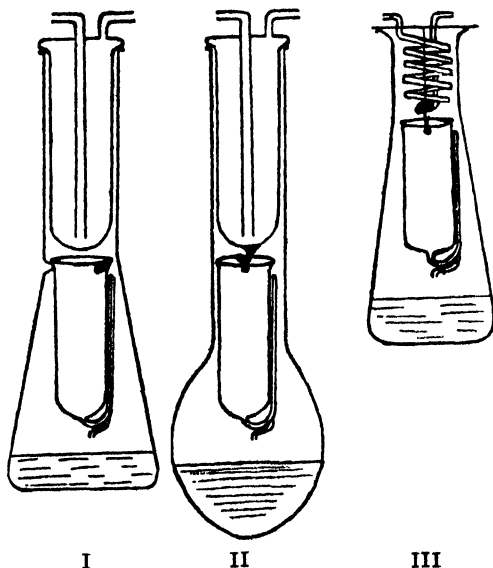
closed so that no air enters *A*, and by raising and lowering *E* a few times the solutions are made to mix. After a period of 10 to 30 minutes (depending on the nature of the amino-substance), the evolved gases are sent into *B* by manipulation of *C*, *D*, and *E*. The dissolved gases are boiled out as before, and passed over into *B*, until rather more than 10 c.c. are collected. *C* is then turned, *E* raised, and the spent solution forced out of *H*. *A* is washed by drawing in and ejecting, successively, three small quantities of water. Eight c.c. of "alkaline permanganate" solution (50 grms. of potassium permanganate and 25 c.c. of potassium hydroxide per litre) are drawn in, care being taken to avoid entrance of air. The gases are then brought back into *A*, and the nitric oxide absorbed, after which the nitrogen is returned to *B* and measured. A blank determination must be made with the nitrite solution. After removal of the permanganate solution, the mercury in *A* may be washed with acid and water.

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### EXTRACTION APPARATUS FOR RUBBER, CELLULOSE, FATS, ETC.

A "SIMPLE all-glass extraction apparatus" was described in 1913 by C. Beadle and H. P. Stevens (ANALYST, 1913, 38, 143). This apparatus was based on a modification of the Soxhlet or Knoefler principle, and was designed to avoid the use of ground-glass joints, corks or mercury seals. In its original form, or in a slightly modified form, in which a round-bottomed flask replaced the conical flask, it has



been in use in these laboratories since that time. In recent years a modification, in accordance with suggestions then made (*loc. cit.*), has been introduced in order to overcome the only disadvantage attaching to the apparatus, namely, the necessity for the transference of the extract for the purpose of evaporation, drying

and weighing. The apparatus in its final form would appear to be generally used on the Continent and in America (G. S. Whitby, *Trans. Inst. Rubber Ind.*, 1925, 1, 18; *Rubber Age (London)*, Oct., 1924; cf. *Elektr. Z.*, 1922, 43, 295, 483), but is seldom seen in this country.

For this reason the following description and drawings, which are self-explanatory, are appended:

Form I is that originally employed, the drawings being approximately a quarter of the natural size. Form II, which is that at present employed, is especially adapted for use on a water-bath. Form III, the improved type, is very similar to Form I except that, as originally suggested by Beadle and Stevens (*loc. cit.*), a more effective condenser (lead or tinned copper coil) has been introduced, thus enabling the total length of the flask to be reduced, so that this may be accommodated on an ordinary balance pan. When removing the solvent after extraction it is, therefore, unnecessary to transfer it to a separate weighed flask, as is the case with Forms I and II.

The advantages of Form III may thus be summarised:—(1) Absence of corks, ground-glass joints and mercury seals; (2) efficient extraction due to adherence to Knoefler principle; (3) general simplicity and compactness of the apparatus, thus giving: (a) Comparative cheapness. (b) Interchangeability of parts should breakages occur; and ready replacements of any part to recomplete the apparatus. (c) No transference of extract necessary. (d) Type of extraction thimble at choice, *i.e.* straight through or with Soxhlet siphon tube. (e) Adaptability to water-bath or hot plate. In connecting up the condensers of several of these pieces of apparatus it is advisable to use thin-walled rubber tubing, so that the weight of the connection has no tendency to upset the flasks.

The apparatus has two limitations: first, the size of the sample that may be extracted is restricted to the capacity of the balance used for weighing the flask; and, secondly, solvents must not be used which might attack the metal of the condenser.

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### CALCULATION OF ALCOHOL FROM THE SPECIFIC GRAVITY.

SOME years ago, when calculating the relations of the various ways of stating alcoholic strength (*ANALYST*, 1919, 44, 167), I assumed, without trial, that the method of calculation of Thorpe's "*Alcoholometric Tables*" was the same as that used by Hefner (*ANALYST*, 1880, 5, 42), and gave values with five or more significant figures.

Hefner, after accepting relationships between specific gravities and percentages of alcohol by weight, used constants to calculate from them the corresponding percentages of proof spirit and alcohol by volume.

I am indebted to Mr. F. C. H. Tate for pointing out that the percentages of proof spirit in Thorpe's tables are obtained by multiplying the percentage of alcohol ( $v/v$ ) at 50° F. by 1.7535. Also, that the ratio of the percentage of alcohol ( $v/v$ ) at 50° F., to that of alcohol ( $v/v$ ) at 60° F. is not constant, and, therefore, the ratio of alcohol ( $v/v$ ) at 60° F. to proof spirit is not constant. The differences are not serious; for example, division of the percentage of alcohol ( $v/v$ ) at 60° F. into the corresponding percentage of proof spirit gives the factor 1.7535 with absolute alcohol, and 1.7482 with 25 per cent. alcohol.



Owing to this fact, calculations assuming constants will have small errors except at one particular strength of alcohol. In the following revised formulae, proof spirit is taken as a convenient mean strength:

$$\text{Per cent.} = \frac{0.7940 v/v}{S} = \frac{w/v}{S} = \frac{0.4533P}{S}.$$

$$v/v = \text{per cent.} \times 1.259S = 1.259 w/v = 0.5711P.$$

$$w/v = \text{per cent.} \times S = 0.7940 v/v = 0.4533P.$$

$$P = \text{per cent.} \times 2.206S = 1.751 v/v = 2.206 w/v.$$

$$S = \text{specific gravity at } 60^{\circ}/60^{\circ} \text{ F.}$$

$$\text{Per cent.} = \text{grms. of absolute alcohol per 100 grms.}$$

$$v/v = \text{c.c. of absolute alcohol per 100 c.c.}$$

$$w/v = \text{grms. of absolute alcohol per 100 c.c.}$$

$$P = \text{c.c. of proof spirit per 100 c.c.}$$

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## Official Appointment.

THE Minister of Health has confirmed the following appointment:

JOHN SINLEY WILSON, as Additional Public Analyst for the County Borough of Burnley (June 16th, 1931).

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### "HOUSEHOLD TURPS."

ON July 10th a retail trading firm was charged at the Salford City Police Court, under Sec. 2 (2) of the Merchandise Marks Act, 1887, with selling a mixture of paraffin and turpentine, to which a false trade description ("Finest Household Turps") had been applied. The manufacturers of the article were also charged, under Sec. 2 (1) of the Act, and with aiding, abetting, counselling, or procuring the trading firm to commit the offence.

The City Analyst (Mr. H. E. Monk, B.Sc., F.I.C.) said that the sample consisted of 30 per cent. of turpentine and 70 per cent. of paraffin of the nature of "white spirit." The fact that the sample was not genuine would be apparent to anyone who was accustomed to deal in such products, from both the smell and the appearance of the bubbles formed on shaking.

In cross-examination the witness said that he had heard of an article called "Household Ammonia," and knew that it contained ingredients other than ammonia, such as soap. He did not consider the description "Household Turpentine" to be on the same footing.

Mr. H. Stout, a pharmacist, gave trade evidence for the prosecution to the effect that he understood "Household Turps" or "Household Turpentine," to mean genuine turpentine and nothing else. The price of turpentine was about four times that of "white spirit."

The secretary of the manufacturing firm said that they had been packing the article for eight years. He considered it equal to, if not better than, turpentine for all household purposes.

The solicitor for the defendants argued that household turps had been supplied, and that there was no standard whereby this article should consist wholly of turpentine. If the magistrate found against him on this point, he must fall back upon the defence that his clients had acted innocently.

The Stipendiary Magistrate (Mr. Percy Macbeth) found the charges against both defendants proved. The retail firm would be fined £15 with £5 5s. costs, and the manufacturers £15 (£7 10s. on each charge) with £5 5s. costs.

## Department of Scientific and Industrial Research.

### REPORT OF THE FOOD INVESTIGATION BOARD FOR THE YEAR 1930.\*

**SECTION A. MEAT.**—An expedition visited New Zealand in September, 1929, to make a comprehensive scientific survey of the trade in frozen mutton and lamb from the abattoir in New Zealand to the wholesale market at Smithfield. A brief account of the ground covered is given. Interesting results have been obtained in the further researches on the analyses of the function of muscle and nerve, and it is shown that the mechanism for oxidising lactic acid is not the same as for synthesising glycogen, since when muscles are frozen so intensely that they do not recover their function on thawing, the capacity for oxidising lactic acids still persists, whereas that for forming glycogen is lost. Heart and nerve tissues are also being studied in order to obtain further light on the mechanism of the injury which freezing occasions. Further work on the water relations in colloidal systems shows that there is very little water bound to the gelatin in dead mammalian muscle, most probably not more than 6 per cent. A paper by J. Brooks (*Biochem. J.*, 1930, **24**, 1379) summarises some of the work on changes in muscle pigment.

**Rancidity in Fats.**—The Kreis test is now applied by dissolving 1 grm. of fat in 2 c.c. of benzene; 1 c.c. of concentrated hydrochloric acid is added, followed, after shaking for 1 minute, by 1 c.c. of a 0.1 per cent. solution of phloroglucinol in ether; shaking is continued for 1 minute and, after centrifuging, the pink aqueous layer is compared with standards. At low temperature the rate of increase of the Kreis reaction is much less than that of the active oxygen content (but in sunlight the opposite occurs), so that rancidity, as measured by any chemical test, may not necessarily bear the same relationship to rancidity measured by taste and odour. Pre-cooling of carcasses for 24 hours at an average temperature of 18.5° C. had no appreciable effect on the keeping qualities of the fat when stored at -5° C., or less, for 30 weeks; a temperature of -10° C. is satisfactory for over 30 weeks, but at -5° C. the growth of moulds and yeasts is not completely inhibited, and sweating caused by exposure to ordinary temperatures for short times

\* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2, pp. 175. Price 3s. 0d. net.

during storage has a bad effect on the fat. The chemical changes occurring in the fat of beef stored at 0° C. have been studied. After 25 days, followed by 4 days at 10° C., the fat was in good condition, but in 42 days a definite taint had appeared in places. The movement of air had a striking effect in reducing the growth of moulds and prolonging the storage life of the fat. A considerable amount of work has been done on the *Actinomyces* of cold stores, which appear to be the only micro-organisms giving rise to a musty odour.

**SECTION B. FRUIT AND VEGETABLES.**—The biochemical study of senescence in apples is not yet complete. The study of the effect of acetaldehyde on the germination of fungal spores (ANALYST, 1930, 55, 509) has been extended to the growth of moulds, and it is found that, although their rate of growth is generally at first inhibited or much retarded, it usually increases with the time of exposure. The age of the colony, temperature, and concentration of the acetaldehyde influence the result. As a whole, volatile substances fall into two classes: those which retard growth to a constant value, *e.g.* chloroform, and those with which "adaptation" occurs, *e.g.* acetaldehyde. The study of the loss of water from apples confirms the view that the mechanism by which water passes from the cell vacuoles to the outer air is complicated, and the rate of loss seems to be governed by the resultant of a number of still not understood internal factors. The work on the action of moderate strengths of cyanide on potatoes at 15° C. shows that changes in respiration are mainly related to the action of cyanide on the sugar content, probably affecting the starch-hydrolysing system.

Investigation of the vitamin content of apples still leaves Bramley's Seedling richer than other varieties, Lane's Prince Albert coming next. Storage in the frozen state does not affect the vitamin content. Vitamin C is at least six times as concentrated in the peel as in the core region. Peas may be preserved without objectionable flavour if they are first cooked for about 8 minutes before freezing in water at 0° F.

**SECTION C. PIG PRODUCTS.**—The freezing and storage of pork and mild-cured bacon, and the scientific basis of canning, including the swelling of gelatin in solutions of sodium chloride, and the effect of sodium chloride on pork muscle are dealt with.

**SECTION D. BIOLOGICAL ENGINEERING.**—The long-felt need of an experimental chamber intermediate in scale between the small constant-temperature room and the immense ship's hold has now been supplied by the erection of a large experimental store at Ditton Laboratory capable of holding some 120 tons of fruit, and an empirical study of the distribution of temperature inside a mass of fruit consisting of 6,000 boxes of apples is being made. The determination of various biological constants for engineering use, particularly in connection with the rate of evaporation from eggs as affected by changes of temperature, humidity and air movement, was continued.

**SECTION E. CANNING.**—See ANALYST, 1931, 56, 315.

**SECTION F. FISH.**—The work on the low-temperature preservation of haddock has been carried further, and, so far as commercial practice goes, the fish may be successfully stored at 29° to 25° F, or -9° to -13° F. or lower. The first temperature has the disadvantage of allowing bacterial growth to proceed, and the second involves greater expense in refrigeration. At intermediate temperatures the "drip" (*i.e.* easily expressible fluid) is most obvious.

Work on post-mortem changes in the liver fat of various fish shows that there are different rates of development of free fatty acids for different species, and that after storage the percentage of free fatty acids developed in the oil of the outer

richer layers of liver is less than in the inner. Lipase is probably evenly distributed in the liver, and the existence of a fat-splitting enzyme other than lipase is suspected.

The effect of bacteria on the spoilage of fish has been investigated, and of the numerous bacteria occurring in the slime on the fish, the group *Achromobacter* is most prevalent, and there was almost complete absence of spore-bearing organisms. The fish muscle was found to be sterile, and of two batches of fish, one untreated, and the other treated with germicidal solution, the muscle of the first group was contaminated several days before the second, which took 7 to 10 days. Invasion from the intestine seems to be an unimportant factor in spoilage. The smoke curing of fish has also received attention, and control of humidity seems to be an important factor.

SECTION G deals with researches conducted at the National Physical laboratory under the direction of the Engineering Committee.

SECTION H. RESEARCHES ON FRUIT.—The chemical work on fruit, at the Imperial College of Science and Technology, has been mainly concerned with the effect of maturity at gathering on subsequent behaviour of apples in cold store. Chemical changes in apples in store at 1° C., gathered at different dates, involved many determinations of fructose and glucose, and it is emphasised that, as stated in the report for 1929, clearing with basic lead acetate and subsequent de-leading, to remove oxidisable substances other than sugar, is an essential preliminary to iodimetric determination of the sugars. If potassium oxalate is used as the de-leading agent, the copper-reducing values are too low, and the iodine values too high (owing to slight oxidation of the oxalate), but if sodium phosphate is used as the de-leading agent, no corrections are necessary. Alcoholic solutions of apple pulp cleared with sodium phosphate, however, develop a yellow colour on standing, and, to obtain concordant results, such solutions should be decolorised by boiling with charcoal before the sugar is determined. Both these reagents are satisfactory with extracts from mature apples, but sodium phosphate only is suitable for very immature apples, owing to the coloured solution obtained with the oxalate. Since analyses with immature apples showed that the alcohol-insoluble residue increased considerably before iodine showed the presence of starch, a direct method for the determination of starch by enzyme hydrolysis was used. In order to obtain the tissue sufficiently disintegrated, some of the pectin was first removed by shaking the material in the cold with potassium oxalate and washing free from oxalate; the hydrolysis was then effected with taka diastase, and the resulting solution of maltose and glucose used for the determination of each sugar, by a combination of the iodimetric method and of Hane's modification of the Hagedorn and Jensen method of oxidation by potassium ferricyanide.

D. G. H.

## THE RELATIVE VALUES OF COD-LIVER OILS FROM VARIOUS SOURCES.

J. C. DRUMMOND AND T. P. HILDITCH.

(*Empire Marketing Board*, No. 15, December, 1930.)\*

PRIMARY SOURCE OF VITAMIN A.—Confirmatory evidence was obtained that the primary source of vitamin A is the minute green plant life of the sea, but there was no evidence as to the formation of vitamin D in these microscopic

\* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 1s. 0d. net.

plants. The small crustacea which feed on these plants, and themselves form a main supply of the cod's food, are curiously deficient in both vitamins, but the balance of evidence favours the supposition that the vitamins are retained by the liver of the cod, and the mature liver is many times richer in them than the foods of the fish. Variations in the vitamin activity of cod-liver oils appear to be wider for vitamin *A* than for vitamin *D*, but, generally, an oil rich in vitamin *A* will be a good source of vitamin *D*, and the nature of the food supply and the quantity of oil stored in the liver appear to be the main causes of variation. The higher the yield of oil from the livers, the lower, relatively speaking, will be the vitamin content, and the richest oils will be obtained from areas where abundant food is available at seasons when the oil content of the liver tends to be low. The Newfoundland area yields the richest oil, followed by Iceland, and then by Scotland, and the Norwegian oils are much poorer.

**ASSAY OF VITAMINS.**—The assay of vitamins in cod-liver oil is unfortunately complicated by the use of three methods: the Norwegian (Poulsen method) giving a value of 250 units, where the British (Pharmaceutical Society assay) gives 100 units, and the American method giving 15 units. In the opinion of the authors, an unfailing indication of the medicinal value of an oil is the intensity of the blue colour produced with arsenic or antimony trichloride, and evidence is accumulating that it is vitamin *A* itself which is responsible for the blue colour. The natural colour of the cod-liver oil tends to run parallel with the vitamin content, and the pale oils from the Lofoten fisheries are, generally speaking, of low vitamin (certainly vitamin *A*) activity. Analyses of a large number of samples of cod-liver oil of known origin are given to elucidate the composition, and include detailed investigations of the fatty acids present in typical oils.

**PREPARATION OF MEDICINAL COD-LIVER OIL.**—In the preparation of medicinal cod-liver oil it is of the greatest possible importance to use absolutely fresh livers, and the oils should on no account whatever have free acidities above 0.5 per cent. The fat-splitting enzymes in the liver must be destroyed, and for this reason a temperature above 70° C. must be reached, and steam at a pressure of 60–100 lbs. is recommended. Insufficient cooking produces oils tending to rancidity on storage. At all stages, contact with air should be reduced to a minimum, but the use of inert gases appears to have little to recommend it. The air-spaces left in the containers should be as small as possible, and, if glass is used, it should be dark amber in colour.

**SUGGESTED STANDARDS FOR COD-LIVER OIL.**—It is suggested that cod-liver oil for human use should have a colour (in 1 inch cell.) of not more than 10 yellow and 0.5 red Lovibond units; (for farm stock, 15 yellow; 1 red); that the free acidity (as oleic acid) should not exceed 0.5 per cent. (1 per cent. for farm stock); that the unsaponifiable matter should not be greater than 1.5 per cent. (the same for farm stock), and that the vitamin *A* reaction should show more than 7 blue units when measured by technique B or C (see below), that is in 10 per cent. dilution (same for farm stock). It is further suggested that containers should bear on the label a statement of the free acidity and response to the colour reaction and the date of the tests.

**VITAMIN A REACTION.**—*Technique B.*—One grm. of oil is accurately weighed and dissolved in 10 c.c. of redistilled chloroform; 0.2 c.c. of this solution is placed in a 1 cm. cell and 2 c.c. of a 30 per cent. solution of antimony trichloride in chloroform are quickly added. The cell is shaken during mixing, and exactly 30 seconds after addition of the antimony solution was begun the colour is matched in a Rosenheim-Schuster tintometer. At least four separate estimations are made, and then

a series of estimations on greater or lesser concentrations, and from the plotted results (which are nearly always linear) the value for a 10 per cent. solution is obtained.

*Technique C.*—The same weight of oil is weighed into a 10 c.c. flask and dissolved in 10 c.c. of the chloroform. The temperature of the solution and that of the antimony trichloride reagent must be between 15° and 17° C. The oil solution is pipetted into the cell, the reagent added as above, and the blue colour matched in the Rosenheim-Schuster tintometer, as before. This is conveniently expressed in the Lovibond scale, and three estimations are made, and a further set at two lower concentrations if the test shows more than 10 blue Lovibond units, or on higher concentrations if it shows less. If the relation is not linear, it may be advisable to separate the non-saponifiable matter, with precautions against oxidation.

D. G. H.

## Connecticut Agricultural Experiment Station.

### REPORT ON FOOD PRODUCTS AND DRUG PRODUCTS FOR THE YEAR 1929.

THIS Report summarises the work done in connection with the inspection of food and drugs, most of the samples being submitted by the Dairy and Food Commissioner. The Department of Analytical Chemistry also tests and issues certificates as to glassware used in carrying out the Babcock test on milk and cream, and of thermometers used in the control of the pasteurisation of milk. The total number of foods examined was 1462, of which 131 were adulterated or below standard, and 17 of 57 drugs were condemned. Investigations of special foods, and of tobacco, have also been made.

**CARBONATED BEVERAGES.**—Only 3 of the 149 samples of the soda water type failed to comply with the requirements. Saccharin was present in 1, artificial colour was not declared in 1, and one sample bore misleading statements as to equivalent food value. The minimum limit for sugar was always exceeded.

**"NEAR BEER."**—Twenty-three samples of malt beverages of the "near beer" type were examined. Two were found to contain caffeine in the proportion of 0.8 and 0.9 grain per 12 oz. bottle. Objection was taken to these, on the ground that caffeine is an added ingredient foreign to the article generally known as beer, which these articles purported to be.

**CEREAL PRODUCTS, ETC.**—Analyses have been given of various cereal breakfast preparations in previous reports (*cf.* ANALYST, 1929, 54, 161; 1930, 55, 129). Eighty-six new analyses have been added, from which the following are quoted:

	Water. Per Cent.	Ash. Per Cent.	Protein. Per Cent.	Fibre. Per Cent.	Carbohydrate other than	Fat. Per Cent.	Calories per 100 Grms.
					fibre. Per Cent.		
<i>Barley Preparations.</i>							
Cream of barley ..	9.2	1.4	11.1	0.6	76.1	1.6	363
Quaker Scotch brand							
pearled barley ..	12.1	1.0	9.5	0.3	76.2	0.9	351

		Water. Per Cent.	Ash. Per Cent.	Protein. Per Cent.	Fibre. Per Cent.	Carbohydrate other than fibre. Per Cent.	Fat. Per Cent.	Calories per 100 Grms.
<i>Corn (Maize) Preparations.</i>								
Cerealine .. ..	11.2	1.5	6.9	0.1	79.9	0.4	351	
Jersey cornflakes .. ..	7.7	0.9	8.5	0.3	82.3	0.3	366	
Post toasties .. ..	11.7	1.8	6.6	0.2	79.4	0.3	347	
Quaker corn puffs .. ..	12.0	0.4	8.7	0.1	78.5	0.3	352	
Quaker homing grits ..	13.2	0.5	7.9	0.2	77.7	0.5	347	
<i>Oat Preparations.</i>								
Keen & Robinson's granulated Scotch oatmeal .. ..	10.4	1.9	13.7	0.8	64.1	9.1	393	
Quaker oats .. ..	10.8	1.9	15.9	0.9	64.5	6.0	376	
Robinson's patent groats	8.4	1.8	12.8	0.7	67.7	8.6	399	
Scotch porage oats ..	10.1	1.7	13.3	0.4	64.9	9.6	399	
<i>Rice Preparations.</i>								
Cook's flaked rice ..	12.6	0.4	7.8	0.2	78.9	0.1	348	
Milk rice .. ..	12.3	3.2	6.9	0.2	77.2	0.2	338	
Quaker puffed rice ..	12.2	0.4	7.6	0.1	79.5	0.2	350	
<i>Rye Preparations.</i>								
Cream of rye .. ..	11.5	1.7	12.0	1.4	71.8	1.6	350	
Ry-krisp .. ..	5.8	2.8	14.0	1.3	74.4	1.7	369	
<i>Wheat Preparations.</i>								
Granose biscuit ..	11.3	3.9	10.3	1.8	71.1	1.6	340	
Force .. ..	10.7	2.8	10.6	1.1	73.7	1.1	347	
Quaker puffed wheat	11.5	1.8	13.1	1.6	70.2	1.8	349	
Triscuit .. ..	10.3	1.7	11.0	1.7	73.9	1.4	352	
Shredded wheat biscuit	8.5	1.5	11.0	2.6	75.0	1.4	357	
<i>Wheat Bran.</i>								
Jireh wheat bran ..	11.1	4.3	16.8	6.3	56.7	4.8	337	
Kellogg's sterilised wheat bran ..	9.6	6.0	16.3	8.5	54.4	5.2	330	
<i>Wheat Bran Biscuits, etc.</i>								
Bran bisque .. ..	8.5	3.1	12.1	2.2	61.0	13.1	410	
Dietetic bran biscuit ..	9.3	5.0	9.9	1.7	69.1	5.0	361	
Fruit nut cereal ..	7.3	3.2	13.5	2.4	72.4	1.2	354	
Laxative biscuit (Kellogg) .. ..	9.4	3.0	16.7	2.4	57.7	10.8	395	
<i>Miscellaneous.</i>								
Grape nuts .. ..	10.3	1.9	11.5	1.5	74.2	0.6	348	
Sea Moss Farina ..	15.6	13.6	9.1	1.5	59.9	0.3	279	
Trix .. ..	6.2	1.5	14.5	0.3	77.3	0.2	369	

"CAFFEINELESS" COFFEE.—A product, sold as "Al-Mo-Co," consisted of a mixture of cereal, coffee, molasses and chicory. It contained 0.18 per cent. of caffeine, and claimed to be 99.74 per cent. caffeineless. It may not be clear to every purchaser that ordinary coffee is about 98.8 per cent. caffeine-free, so that this product contained about one-seventh of the amount of caffeine in ordinary coffee.

**SPECIAL FOODS.**—Analyses were made of 54 special and miscellaneous foods, including some for diabetic patients. Of the articles included in the following table, Nouron is made from soya beans, whole wheat flour and egg yolk, and "Fiddle heads" are a species of native ferns said to have been used by the Indians as food.

	Water. Per Cent.	Ash. Per Cent.	Protein (N × 6.25). Per Cent.	Fibre. Per Cent.	Carbohydrate.		Undeter- mined. Per Cent.	Fat. Per Cent.
					Starch + Water- soluble (as dextrose). Per Cent.	Undeter- mined. Per Cent.		
Flour of cooked chestnuts	6.30	2.00	6.63	2.05	65.28	14.76	2.98	
Loeb's gluten breakfast cereal .. ..	6.18	3.12	37.28	0.98	27.44	8.07	16.93	
Mellin's food .. ..	2.20	3.90	10.63	none		81.54	1.75	
Nouron .. ..	8.30	2.48	24.38	3.05		52.46	9.33	
"Flour of Algae" ..	7.48	35.62*	5.38	7.43		43.46	0.63	
Soy cheese .. ..	77.20	0.55	14.44	—	trace	4.31	3.50	
Fiddle heads ( <i>Osmunda cinnamomea</i> ) ..	87.03	1.24	4.72	1.04		5.56	0.41	

\* Total  $P_2O_5$ , 0.60;  $Fe_2O_3$ , 0.24; CaO, 1.75; iodine, 0.15 per cent.

**ICE CREAM.**—Of the 301 samples submitted, 4 were unsatisfactory. The State standard for fat content in plain ice cream is 8 per cent., and for fruit and nut ice cream 6 per cent. A Federal standard, fixing the fat limit for plain ice cream at 12 per cent., has never become official.

**CIDER VINEGAR.**—The law requires that vinegar (cider vinegar) shall contain not less than 1.6 per cent. of total solids. The Federal standard has been revised, so that the only numerical standard is that for acid strength (4 per cent.). Since it is now known that genuine vinegar may sometimes contain less than 1.6 per cent. of total solids, it may be unfair to adhere strictly to that limit.

**TOBACCO SEED.**—In addition to 167 partial analyses of tobacco, proximate analyses of tobacco seed and of fresh and cured leaves were made. The tobacco analyses gave the following results:—Water, 3.34; ash, 3.71; protein (N × 5.34), 20.76; fibre, 14.44; carbohydrate other than fibre starch, none; water-soluble after hydrolysis (as dextrose), 3.08; water-insoluble after hydrolysis (as dextrose), 0.55; undetermined carbohydrate, 11.89; fat (etheral extract), 42.23 per cent.

The fresh and cured leaves contained 1.88 and 1.51 per cent. of starch, calculated on the air-dry substance. The starch was determined by hydrolysis of the material with malt, after successive extractions with ether, alcohol, and water.



## International Atomic Weights.

### FIRST REPORT OF THE COMMITTEE ON ATOMIC WEIGHTS OF THE INTERNATIONAL UNION OF CHEMISTRY.\*

THE report comprises investigations published since January 1st, 1930. The atomic weight of nitrogen was calculated from specific gravity determinations of ammonia gas as 14.009; the specific gravity of phosphine also was determined, giving  $P = 30.977$ . Synthesis of silver sulphide furnished the value  $S = 32.066$ . The atomic weight of calcium was checked by chlorine determinations on anhydrous calcium chloride fused in hydrogen chloride and cooled in nitrogen; average, 40.085. The value 50.948 for vanadium almost agrees with the present figure. Determination of chlorine in chromyl chloride gave the figure  $Cr = 52.02$ ; arsenic trichloride, also analysed for chlorine, led to  $As = 74.938$ . Halogen determination in tantalum chloride and bromide resulted in a lower figure, namely,  $Ta = 181.36$ . The atomic weight of rhenium, 186.31, as determined by analysis of silver perrhenate prepared by three different methods, is 2.4 units lower than Noddack's provisional figure. Thallous bromide was analysed, the result ( $Tl = 204.390$ ), confirming the earlier one.

A revised complete table of atomic weights is appended; such alterations as occur affect only the second decimal place. The following figures are of interest:  $Re = 186.31$ ;  $Ta = 181.4$ ;  $Ti = 47.90$ ;  $Zr = 91.22$ .

W. R. S.

\* By G. P. Baxter, Mme. M. Curie, O. Hönlgschmid, P. Le Beau, and R. J. Meyer. (*J. Amer. Chem. Soc.*, 1931, **53**, 1627-1639).

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Chemical Changes in the Fat of Frozen and Chilled Meat. Part I. Frozen Mutton and Lamb.** C. H. Lea. (*J. Soc. Chem. Ind.*, 1931, **50**, 207-212T.)—To follow the progress of oxidation a very thin layer of surface fat, 3 to 4 sq. in. in area was removed from the breast of the carcase, frozen at  $-20^{\circ}C$ ., trimmed at  $0^{\circ}C$ . until only a very thin layer (about 0.5 mm.) of fat-impregnated connective tissue remained. This material was crushed and cut up finely, 1 gm. weighed into a test tube, and the active oxygen content determined, using 19 c.c. of glacial acetic acid and chloroform mixture (*Proc. Roy Soc.*, 1931, **3**, 108, 175, and Reports of the Food Investigation Board, ANALYST, 1930, **55**, 570; 1931, **56**, 531). Free acidity was determined by the direct titration method of Pennington and Hepburn for chicken fat (*J. Amer. Chem. Soc.*, 1910, **32**, 568). The fat (10 grms.) was cut up, ground at  $0^{\circ}C$ . with silver sand (1 gm.), boiled with 95 per cent. alcohol (100 c.c.) for 5 minutes for kidney fat and 10 minutes for breast fat, and titrated to phenolphthalein with 0.05 N potassium hydroxide. The changes

in the free acidity and superficial oxidation in the fat of carcasses of lamb were followed during pre-cooling, cold storage at various freezing temperatures, and at ordinary temperatures subsequent to storage. The maximum values reached for free acidity were about 1.2 per cent. (as oleic acid) for external fat, and rather less for kidney fat; 24 hours' pre-cooling at the ordinary temperature gave rise to no signs of superficial oxidation, and no definite increase in susceptibility to oxidation during subsequent storage. The fat of carcasses held at  $-5^{\circ}\text{C}$ ., or below, for periods up to 7 months, showed no oxidation sufficient to affect the flavour after 3 days' exposure to ordinary temperatures in not too strong a light. Sweating only exerts a markedly deleterious effect on the keeping properties of the fat when held subsequently at ordinary temperature if it has occurred in the presence of light; but even for short periods it produces a bad effect on the appearance of the carcass, although the lower the temperature of storage the less the bad effect of exposure for short periods to temperatures above  $0^{\circ}\text{C}$ . No visible growth of moulds or yeasts occurred at storage temperatures of  $-10^{\circ}\text{C}$ . or  $-20^{\circ}\text{C}$ ., but growth was not inhibited at  $-5^{\circ}\text{C}$ . In no case did micro-organisms appear to be present in the fat when the meat was held for 3 days after storage at  $12^{\circ}\text{C}$ .

D. G. H.

**Two New Sugars of Human Milk, Gynolactose and Allolactose.** M. Polonovski and A. Lespagnol. (*Compt. rend.*, 1931, 192, 1319-1320.)—The divergent results obtained on determining the sugar in the serum of human milk by the ordinary methods of reduction and polarimetry are due to the presence of sugars other than lactose. Two such sugars have now been isolated by means of a long series of fractional crystallisations from water and from methyl and ethyl alcohols. (1) *Gynolactose*, readily soluble in water, but almost insoluble in ethyl alcohol, has m.pt. about  $205^{\circ}\text{C}$ . and, in 3 per cent. aqueous solution,  $[\alpha]_D -27^{\circ}$ . It shows no apparent mutarotation, and its reducing power, as given by Bertrand's method, is about 40 per cent. of that of dextrose. It yields no phenylosazone, and gives galactose (50 per cent.) and dextrose on hydrolysis by strong acids. (2) *Allolactose*, also highly soluble in water, has m.pt.  $165^{\circ}\text{C}$ . and  $[\alpha]_D +20^{\circ}$  in aqueous solution. It exhibits slight and extremely transitory mutarotation. Towards iodine it behaves like an aldobiase, and its reducing power is about two-thirds of that of dextrose. The phenylosazone, which dissolves in boiling water, crystallises in mammillary masses of radiating structure and has the instantaneous m.pt.  $172^{\circ}\text{C}$ . This sugar also furnishes dextrose and galactose on hydrolysis. T. H. P.

**Evaluation of Honey on the Basis of the Diastase Content.** K. Braunsdorf. (*Z. Unters. Lebensm.*, 1931, 61, 411-420.)—Of the 62 German honeys examined, one had a diastatic value of 62.5; 18 of 23.8 to 50; 18 of 11 to 18; and 8 less than 10; where the value was medium to low the phloroglucinol test indicated strong heating (*cf.* Fiehe and Kordatzki, *ANALYST*, 1928, 53, 388). The value 23.8 is, therefore, suggested as a lower limit for first-class diastatic honeys, with 17.9 as the lowest permissible limit for unheated honeys and 10 for honeys assessed on the basis of the (1930) German Regulations (*cf.* following abstract, and

Braunsdorf, *Z. Unters. Lebensm.*, 1930, **60**, 575). On the whole, there was correspondence between the Fiehe and phloroglucinol tests (*cf. loc. cit.*), but the latter does not readily distinguish between unheated genuine honeys having a naturally low diastatic power and those the diastase of which has been crippled by heating. This is important, since the effect of heat is greatest for diastatic values of about 11 to 14. Absence of colour change, or a change to pale yellow on addition of the reagent in the phloroglucinol test, is an indication of unheated or slightly heated honey (*i.e.* natural diastase is present), whilst a yellow-gold colour indicates weakening of the diastase due to strong heating. It is stated that, as a rough guide for this test, a honey of fairly high diastatic value heated for 1 hour at 60 to 65° C. gives a colour (fading after 5 minutes) similar in shade and intensity to that of a 0.1 *N* solution of potassium dichromate. J. G.

**Honey Diastase.** J. Fiehe. (*Z. Unters. Lebensm.*, 1931, **61**, 420–427.)—The diastatic powers of 190 German and foreign honeys were determined by Fiehe and Kordatzki's modification of Gothe's test (*ANALYST*, 1928, **53**, 388) before and after melting at 60° C. in a steam-heated vessel provided with a stirring apparatus. Under these conditions the lowering of diastatic value is small and, in many cases, nil; the value was reduced to 8.3 in only 12 cases, and to less than 8.3 (to 5 and 6.5) in 2 cases, and 8.3 is, therefore, considered a suitable lower limit of diastatic power, and is becoming recognised as such in the trade. Attention is directed to certain Californian honeys which are naturally poor in diastase, and which cannot, therefore, be said to contravene the German Regulations, March, 1930, since these stipulate that the low diastatic value must not be the result of heating. Analyses of two such honeys ("orange-blossom" honey having a crystalline consistence, pale yellow colour and orange-blossom odour, and "belvedere" honey, crystalline, bright yellow, with the odour of alfalfa or lucerne honey) gave, respectively:—Water, 16.75 and 11.50; invert sugar, 72.84 and 76.03; sucrose, 1.07 and 3.90; glucose, 35.65 and 38.08; fructose, 37.19 and 37.95; ash, 0.04 and 0.052 (percentages); acidity, 1.2 and 0.84 c.c. of 0.1 *N* sodium hydroxide solution per 100 grms.; diastase value, 2.5 and 1.0. The end-point in the above diastase test may be sensitised by addition to the tubes concerned of a little aluminium hydroxide, precipitated from alum by ammonia and washed. This adsorbs any blue colour, which may thus be distinguished from the purple of the iododextrins remaining in solution (*cf.* preceding abstract). J. G.

**Determination of the Acidity of Red Wines by means of Fluorescent Indicators.** Y. Volmar and S. M. Clavera. (*J. Pharm. Chim.*, 1931, **13**, 561–568.)—Titration of red wines in presence of litmus or phenolphthalein gives high values for the acidity. By the use of the fluorescent indicator, umbelliferone, results agreeing closely with the theoretical acidity, as determined by potentiometric titration, are, however, obtainable. Five c.c. of the wine, freed from carbon dioxide by agitation in a vacuum, are diluted to 100 c.c., and the solution transferred to a thin glass or, preferably, Pyrex Erlenmeyer flask, and treated with from 2 to 5 drops of alcoholic umbelliferone solution of 1:100,000 concentration.

The flask is placed in a dark room, in the path of the rays from a silica mercury-vapour lamp of Westinghouse type, working at 110 volts. This lamp is enclosed hermetically in a box furnished with a window of Wood's nickel oxide glass, which allows of the passage of only ultra-violet light of wave-length above  $0.35\mu$ . While exposed to this light, the liquid is titrated with 0.1 or 0.05 *N* sodium hydroxide until it just exhibits blue fluorescence. If acridine is used in place of umbelliferone, the initial green fluorescence changes suddenly to indigo-blue on titration, at pH 4.9, so that this indicator gives correct results only if a suitably-adjusted solution is used for comparison purposes. These indicators are useless in the determination of the acidity of beer, owing to the excessive intensity of the fluorescence of this beverage under Wood's light.

T. H. P.

**Systematic Examination and Evaluation of the Kreis Reaction. K. Täufel.** (*Chem. Ztg.*, 1931, 55, 434.)—For normal fats the Kreis test provides a very sensitive indication of the beginning of the series of reactions producing rancidity, phloroglucinol being preferable as a reagent to resorcinol or naphthoresorcinol. The diethyl and glycol acetals of the unstable epihydrinaldehyde gave reactions in solutions corresponding with 0.5% of aldehyde per c.c. This aldehyde, which has been suggested as an intermediate product of the rancidity reaction, has a slight stability in acid or neutral solution, but decomposes rapidly in an alkaline medium. The Kreis reaction may be used as a test for epihydrin aldehyde, but the interference of fatty substances should be eliminated by directing a jet of steam containing the vapour of the aldehyde against a wad of wool or lignin-free paper which has been soaked in a mixture of an ethereal solution of phloroglucinol and concentrated sulphuric acid.

J. G.

**Glyceride Structure of Butter-Fats. T. P. Hilditch and J. J. Sleight-holme.** (*Biochem. J.*, 1931, 25, 507–522.)—Four English butter-fats from members of the same herd of cows fed on various diets and a New Zealand pasture-fed (December) butter-fat have been oxidised in order to determine their content of fully-saturated glycerides, and the fatty acids of the latter have been analysed in detail. The results obtained, with similar data for two other New Zealand butter-fats and an Indian cow ghee, are discussed with reference to the general glyceride structure of butter-fats (*cf.* ANALYST, 1931, 161). The six butters from cows on "normal" (usually pasture-fed) diets show many features in common, but in two cases in which specific fatty oils had been given to the cows the results stand apart in certain respects; all eight fats conform to the usual rule (in animal fats; *i.e.* beef and mutton tallow, lards, rabbit-fat) that the content of fully-saturated glycerides is a function of the proportion of the total saturated acids present in the mixed acids of the whole fat, irrespective of the nature of the saturated acids (the unsaturated acids are throughout almost constant in composition, *viz.* about 90 per cent. oleic and about 10 per cent. linoleic). In the "normal" butters, the component acids of the fully-saturated parts are present in approximately the same proportions, the composition of the fully-saturated

glycerides tending to be the same whatever the original unsaturation of the butter as a whole. Similar concordances are shown in the combined butyric-lauric acid contents, and in the combined myristic-palmitic acid contents of the whole fats and of their two divisions, fully- and non-fully-saturated glycerides, but the content of unsaturated acids in the non-fully-saturated glycerides increases steadily and slowly with increase of unsaturation in the fats as a whole. When coconut-fat (a relatively saturated fat) formed part of the diet, the fully-saturated part of the butter showed a marked increase in butyric-lauric acid content, but the composition of the non-fully-saturated glycerides was normal. With a soya bean (predominantly unsaturated) oil diet the structure of the butter glycerides was different; the fully-saturated components were almost normal in composition, but the lower acids of the non-fully-saturated portion were present in excess of the usual proportion. Study of the glyceride structure of these eight butters confirms the general rules which have been observed to connect the amount of fully-saturated glycerides with the degree of unsaturation of animal fats, but it also reveals influences due to diet more clearly than the consideration of the component fatty acids of the whole fats, and affords some additional evidence as to the mode of utilisation of ingested fat in milk-fat metabolism.

P. H. P.

**Detection of Riegel's "New Preservative for Pumpnickel." P. Weinstein, J. Muesmann and W. Bodschwinna.** (*Z. Unters. Lebensm.*, 1931, **61**, 436-442.)—"Antibacterin" and "Mikrobin-P," which have been suggested as preservatives for bread, were identified as sodium *p*-toluene sulphochloramine (also known as chlorazene, miamine and chloramine-T), and benzoic acid containing 10 per cent. of chlorobenzoic acid, respectively. The former is detected in 400 grms. of crustless bread by reducing it to fine crumbs and heating it under a reflux condenser for 15 minutes with 500 c.c. of ether containing 2 c.c. of fuming hydrochloric acid. After filtration the residue is washed with 200 c.c. of ether, the total filtrates evaporated to 50 c.c., the extract transferred to a large dish, and the last traces of ether removed. Fat is then eliminated by extraction with hot 50 per cent. alcohol, and, after filtration on kieselguhr, the residue is washed with two 10 c.c. portions of hot alcohol, and the combined extracts evaporated. (Benzoic acid may separate in feathery masses at this stage.) The residue is extracted with 25 c.c. of warm ether, the mixture filtered into a 50 c.c. cylinder, and the residue washed with warm ether until the 50 c.c. mark is reached. The filtrate is then divided into 25 c.c. portions, which are evaporated, and the respective residues are used for the (Kjeldahl) nitrogen and sulphate determinations. In the latter case the residue is gently fused with a powdered mixture of 2 grms. of dry sodium carbonate and 1 gm. of sodium nitrate, the melt is extracted with water, 10 c.c. of fuming hydrochloric acid added, and the sulphate precipitated with barium chloride. The Mikrobin-P is detected in the ethereal extract by the usual method. Blank tests on preservative-free bread gave no barium sulphate and 7 mgrms. of nitrogen by the method described. Experiments in which known amounts of the preservatives were added to the unbaked meal and the final loaf

analysed led to the conclusion that more than 15 mgrms. of barium sulphate and 10 mgrms. of nitrogen per kilo. in the test indicate the addition of antibacterin.

J. G.

**Trigonelline Content of Coffee.** F. E. Nottbohm and F. Mayer. (*Z. Unters. Lebensm.*, 1931, 61, 429-435.)—Determinations of trigonelline by the authors' method (*ANALYST*, 1931, 56, 405) gave values between 0.228 and 0.245 per cent. for all ordinary trade coffees, whilst in certain "wild" African coffees only 0.1 to 0.2 per cent. was found, such values being associated with a relatively high caffeine content (1.2 to 2.2 per cent.). Surinam-Liberian coffee gave the lowest value obtained (0.11 per cent.). In such cases the lead precipitate of the alcoholic extract (*loc. cit.*) was pure yellow, whilst for normal coffees it is orange-red. There is no evidence of loss or decomposition of trigonelline after roasting at 250° C., but the precipitate with iodine and potassium iodide (*loc. cit.*) is oily in character, probably on account of the presence of basic nitrogenous decomposition products produced by heat. The "alkaloid value," given by the ratio caffeine:trigonelline content is constant (4.3 to 5.5) for all ordinary coffees, but is high (9.7 to 13.7) for wild (*e.g.* Liberian) coffees; treated coffees contain the normal quantities of trigonelline. Aqueous infusions of coffee contain only about 82 per cent. of the total caffeine and 70 per cent. of the trigonelline, so that in such cases it is necessary, if possible, to determine these alkaloids both in the extract and in the residual grounds. The double platinum salt of trigonelline ( $C_7H_7NO_2$ , HCl) $PtCl_4$  exists in only one form (*cf.* the gold salt, *loc. cit.*), *viz.* in anhydrous dense orange-red prisms, m.pt. 215° C.

J. G.

**Colloidal Iodine Preparations.** S. J. Hopkins. (*Pharm. J.*, 1931, 126, 538.)—Some commercial colloidal iodine preparations, paler in colour than usual, were found to be acid to litmus and to give no colour with starch mucilage, and no colour could be removed by chloroform or carbon tetrachloride, but addition of chlorine water was followed by liberation of iodine. The average composition was: Iodine (in combination), 0.21; sodium chloride, 0.74; gelatin, 0.35; and water, 98.70 per cent.; and the acidity (as hydriodic acid) agreed with the amount of iodine present. It was found that when finely divided iodine was heated with a solution of 0.74 per cent. of sodium chloride and 0.35 per cent. of gelatin under pressure in an autoclave, combination occurred, with complete loss of colour and production of acid, so that in these preparations the iodine was not in the colloidal state, nor was the gelatin a protective colloid.

D. G. H.

## Biochemical.

**Effect of Desiccation upon Nutritive Properties of Egg-White. II.** M. A. Boas Fixsen. (*Biochem. J.*, 1931, 25, 596-605.)—It was previously shown by Boas (*Biochem. J.*, 1927, 21, 712) that dried egg-white, when used as the sole source of protein in a diet for young rats, produces a train of characteristic symptoms—notably dermatitis, baldness and spastic gait terminating in death in from 4 to 6 weeks, and that this syndrome could be completely prevented if certain substances

such as yeast, potato, fresh egg-white and others were added to the diet, and the presence of an unknown protective dietary factor X in these substances was postulated. It was suggested (a) that dried egg-white is lacking in some essential dietary factor which is supplied by protective factor X, or (b) that a toxic substance is formed in dried egg-white as a result of desiccation, and that this is neutralised in some way by protective factor X. As there is no prospect at present of continuing this work, which has been carried a stage further but is still incomplete, the results so far obtained are now published. Some of the different constituents of egg-white, fresh or dried, were investigated as the source of protein both by curative and by preventive tests. It is shown that pure ovalbumin, prepared by crystallisation from crude egg-white, forms a satisfactory protein for young rats. The same is true of ovoglobulin and of the total albumin and ovomucoid fraction. Desiccation does not alter the nutritive properties of these materials as is the case with crude egg-white. Therefore, the alteration in nutritive value observed when egg-white is dried is probably due, not to the loss of some essential factor, but to the formation of a toxic substance. The evidence suggests that the toxic substance is formed from some non-protein constituent of the egg-white. A protein fraction uncontaminated by this toxic substance can be prepared from dried egg-white. The protective factor X, capable of neutralising the toxic substance in dried egg-white, is present in raw liver, and also in a dried preparation of the total albumin and ovomucoid fraction of egg-white, but not in raw beefsteak. It is only partly extracted from yeast by boiling dilute acetic acid. It is not removed from caseinogen by washing with dilute acetic acid and extraction with alcohol. Rats suffering from the effects of the dried egg-white can become spontaneously refected in a similar manner to that observed by Fridericia and his colleagues (*J. Hyg.*, 1927, **27**, 70), and by Roscoe (*J. Hyg.*, 1927, **27**, 103) in the case of rats fed upon diets devoid of water-soluble B vitamins. This condition can be transmitted from one rat to another by ingestion of the faeces (bulky white faeces containing undigested starch). Refection can be induced in rats receiving the dried egg-white diet, by feeding them with the bulky white faeces of refected rats on diets deprived of B vitamins. This suggests that the agency is the same in both cases. The facts afford little support to the idea of Findlay and Stern (*Arch. Dis. Childhood*, 1929, **4**, 104) that the egg-white syndrome is identical with "pink disease" (Swift's disease) in children.

P. H. P.

**Effect of High-Pressure Hardening of Oils for the Margarine Industry.** J. A. Van Dijk, R. T. A. Mees and H. I. Waterman. (*Chem. Weekblad.*, 1931, **28**, 319-320.)—It has been shown that it is possible to harden fatty oils by using a nickel catalyst at temperatures of 30 to 60° C. if hydrogen at very high pressure is used, and that with such low temperatures there is much less loss of important constituents. The carotene in palm oil hardened in this way is not destroyed, and cod-liver oil will still show the blue coloration in Carr and Price's reaction. It seems probable that the activity of vitamins may thus be retained in hardened oils, and biological tests are to be made with oils hardened at these low temperatures.

D. G. H.

**Characterisation of Vitamin A. Part I, Spectroscopic Evidence.**

**I. M. Heilbron and R. A. Morton. Part II, Biological Experiments. B. Ahmad and J. C. Drummond.** (*J. Soc. Chem. Ind.*, 1931, 50, 183T-186T).—

It has already been shown with liver oils and concentrates that quantitative determination of the intensity of absorption at  $328m\mu$  (maximum) provides a criterion of vitamin A potency, and that the blue colour obtained with the antimony trichloride test for vitamin A is complex in nature, being the resultant of two absorption bands with maxima at  $572m\mu$  and  $606m\mu$  in fish-liver oils (displaced to  $583m\mu$  and  $620m\mu$  in concentrates). With the idea of establishing definitely if the chromogenic property of liver oils is in the same molecule as the entity giving rise to the  $328m\mu$  maximum, the physical measurements have been extended to embrace as wide a range of liver oils and concentrates as possible. Various discrepancies have been disclosed. The most striking anomaly, from the point of view of the colour test, was the occasional appearance (in relatively fresh oils) of the  $572m\mu$  maximum at an intensity great enough to mask the  $606m\mu$  band almost completely. Re-examination of these oils after several months disclosed a remarkable spontaneous increase in the intensity of the  $606m\mu$  band, so that the oils appeared normal. In spite of this striking change, which could be induced artificially, neither the ultra-violet maximum nor the  $572m\mu$  colour band had undergone significant alteration. The authors had already concluded that a closer correlation, both in oils and concentrates, existed between the  $328m\mu$  maximum and the absorption at  $572m\mu$  ( $583m\mu$  in concentrates) than between the former and the  $606m\mu$  ( $620m\mu$  in concentrates) band, in spite of the customary predominance of this longer wave-length maximum. The experiments on induced or spontaneous oxidation, concerned as they are with very extreme deviations, have led definitely to the view that, whilst the  $328m\mu$  band and the  $572m\mu$  chromogen cannot be differentiated, the  $606m\mu$  chromogen must be a distinct and separate entity, which, although not vitamin A, cannot be rejected as unrelated, and may well provide an indication of vitamin potency which is approximately valid in practice though not in principle. There is probably a fairly close connection between the two chromogens. It is suggested that the most promising criteria for vitamin A will be a colourless, or almost colourless, substance, exhibiting (a) an absorption band without fine structure and with a maximum at  $320-330m\mu$ , and (b) giving with antimony trichloride a sharp absorption band at about  $583m\mu$ , the intensity of absorption for both bands being approximately the same. The reduction product of carotene prepared by Smith (*J. Biol. Chem.*, 1931, 90, 597), and claimed to be "dihydrocarotene," has been prepared and tested. It approaches in its characteristics these criteria for vitamin A, but fails to satisfy them completely, and is not a pure single substance. When it was tested biologically on rats negative results were generally obtained, although one or two cases of irregular growth occurred. These results are not in agreement with those of Karrer, Euler, Hellström and Rydbom (*Svensk. Kem. Tidsskr.*, 1931, 43, 105). Possibly only a small proportion of the "dihydrocarotene" preparation is physiologically active, and the irregularities are due to differences



in the amount absorbed. An investigation of the absorption of carotene from the mammalian intestinal tract and of the mechanism of the conversion of the pigment into vitamin *A* in the tissues is in progress. The results so far obtained are given. It is noted that the addition of fat to the diet improves absorption considerably. The experiments suggest that a large proportion of the carotene absorbed into the organism may be rapidly transformed into other substances, but that the formation of vitamin *A* is relatively a slow process, possibly concerning quite a small proportion of the assimilated pigment. It is highly probable that the breakdown of the complex, unsaturated molecule (carotene) in the organism would follow several paths; reduction might be one of the changes involved, but it must be remembered that there are grounds for the belief that vitamin *A* contains hydroxyl groups.

P. H. P.

**Critical Study of the Antimony Trichloride Colour Test for Vitamin A.**

W. R. Brode and M. A. Magill. (*J. Biol. Chem.*, 1931, 92, 87-98.)—There is much difference of opinion with regard to the specificity of the antimony trichloride colour reaction for vitamin *A*; a systematic study has now been made of the absorption spectra of the solutions with the use of a Bausch and Lomb spectrophotometer to which a modified Duboscq colorimeter was attached. This spectrophotometer permitted either a qualitative examination over a wide portion of the spectrum to determine the position of the band, or a quantitative determination, within a narrow portion of the spectrum, of its intensity. It has been shown that the antimony trichloride colour solutions in the test for vitamin *A* may have two different absorption bands, one at  $578m\mu$  and the other at  $608m\mu$ . Both of these bands fade and the respective solutions develop new bands at  $472$  and  $532m\mu$ . Conditions of concentration have been determined for a few commercial samples of cod-liver oil by which only one of the two bands is produced ( $608m\mu$ ). A saturated solution of antimony trichloride in anhydrous chloroform gave the best results. Under the conditions described cod-liver oils yield extinction coefficient values of the  $608m\mu$  band which are proportional to the concentration of the oil. The procedure adopted for the analysis of oils is as follows:—Five c.c. of saturated antimony trichloride solution are placed in a spectrophotometric cell with 1 drop of acetic anhydride (to react with hydrochloric acid, water, etc., present). Then 0.5 c.c. of a chloroform solution of the oil to be tested is added to form a layer on top of the reagent, the cell is shaken, and the intensity of the  $608m\mu$  band is observed exactly 20 seconds after mixing. A reading is made at  $578m\mu$ , to note if there is a band there; if so, the oil is too concentrated, and a more dilute solution should be used. As a check, another solution of oil of a different concentration is made and observed. The readings of the  $608m\mu$  band should be proportional to the amount of oil used. A 10 per cent. solution of oil should be tried first. If the extinction coefficient of the  $608m\mu$  band for 2 cm. cell thickness is less than 1.50 to 1.75, the check solution may be 20 per cent.; if greater than 1.75, but less than 2.00 to 2.50, 15 per cent.; if greater than 2.00 to 2.50 the check solution should be 7 or 8 per cent. There appears to be a definite relation between the extinction coefficient values of the blue solution ( $608$  and  $578m\mu$  bands), and the faded

or red solutions (532 and 472 $m\mu$  bands). Results will be published in a subsequent paper on the comparisons of results obtained by this method with those obtained by biological assay.

P. H. P.

**Photographic Effects of Vitamins A and B.** S. Botcharsky and A. Foehringer. (*Nature*, 1931, 127, 856.)—These two vitamins are capable of affecting photographic plates, the effects produced being similar in character, in spite of the different origins of the vitamins. An ethereal extract of dried ox-liver, subsequently freed from solvent in an atmosphere of nitrogen, was used as the source of vitamin A, and an aqueous extract of purified brewery yeast as that of vitamin B. The photographic plates were covered with aluminium foil and letters were cut out of the foil covering the glass side. Letters were then painted on the same side with the vitamin extracts, which had been previously tested biologically. After being left for 3 days wrapped in black paper, the plates were developed, clear images of the letters being obtained. Similar images are formed by the vitamins sealed in separate glass tubes. If the vitamins are destroyed, but not carbonised, no effect is produced on the plates.

T. H. P.

**Isolation of the Antineuritic Vitamin.** A. Seidell and V. Birckner. (*J. Amer. Chem. Soc.*, 1931, 53, 2288–2295.)—The preparation of the “activated solid” is the first step in the process of production of the vitamin itself, and for this purpose 160 kilos. of pressed brewer’s yeast (equivalent to about 45 kilos. of dried yeast) are quickly added to about 300 litres of rapidly stirred water at 80° C. After slight cooling, the mixture is filtered and 15 kilos. of fuller’s earth are added, after which the whole is stirred for 1 hour and left for 2 hours for subsidence of the activated fuller’s earth. The supernatant liquid is decanted, and the “activated solid” washed with slightly acidified water (1 c.c. of concentrated hydrochloric acid per litre). The extraction and concentration are effected by vigorously agitating the activated solid in 0.4 to 0.5 *N* sodium hydroxide solution for 5 minutes, and separating the alkaline liquid as quickly as possible by means of a Sharples super-centrifuge. The extract is quickly acidified with sulphuric acid and adjusted to a *pH* of 3.0, this being indicated by the separation of a light fluffy precipitate. The slightly acid extract is distilled under reduced pressure to about a tenth of its volume, and the brownish material which separates is removed by deposition in a large cup centrifuge. The nearly clear supernatant solution is seeded with sodium sulphate decahydrate and kept cool for crystallisation of the large excess of the salt. The brown material is very rich in the thermo-stable growth factor ( $B_8$  or  $G$ ) required as a supplement to the anti-neuritic vitamin, and, when dried, is about five times as active as yeast. The solution from which the excess of sodium sulphate crystallises is treated with about an equal volume of methyl or ethyl alcohol to precipitate more of the inorganic salts and organic impurities present. By sacrificing some of the vitamin, much material interfering with subsequent purification may be eliminated at this stage. The clear, approximately 50 per cent. solution, usually contains about 30 grms. of dissolved solids per litre, of which about 30 per cent. are inorganic salts. The alcoholic solution is distilled

down in separate portions and benzoylated, and the vitamin salts are extracted by rotating for 24 hours with a mixture of 3 volumes of normal propyl alcohol and 1 volume of concentrated hydrochloric acid, using about 3 c.c. of this mixture for 1.0 grm. of salts. This extract is separated by centrifuging and the residue extracted again. The two extracts are then distilled under reduced pressure to about 50 c.c., the liquid centrifuged, and the clear solution added, drop by drop, to about 1600 c.c. of acetone. The resulting precipitate is separated by centrifuging, dissolved in about 15 c.c. of methyl alcohol, and the solution added, drop by drop, to about 800 c.c. of acetone. The white flaky precipitate is centrifuged, washed with acetone, and dried in a vacuum. The activities of 16 precipitates obtained in this way were tested by M. I. Smith's rat method (*Public Health Reports*, 1930, 15, 116), and the most active was curative in 0.03 mgrm. doses, *i.e.* an activity about one-fourth greater than that of the Jansen and Donath crystals. Apparently, small variations of conditions at certain stages, such as the proportion of sodium carbonate used in the benzoylation and differences of temperature, greatly affect the quantity and quality of the final product.

D. G. H.

**Vitamin Studies. III, Vitamin Content of Fruits.** F. V. v. Hahn. (*Z. Unters. Lebensm.*, 1931, 61, 369-411.)—Growth experiments with guinea pigs (full details of which, including weight-curves, are given in the original) indicate that the 40 types of fruit examined may be classified according to their vitamin-C contents as follows:—*Very rich*: strawberry, orange, lemon; *rich*: gooseberry, raspberry, hips (dried and cooked); *vitamin-containing*: red currant, mandarin; *poor in vitamin*: freshly picked apple, morella cherry, greengage, plum (various kinds), peach, banana, pineapple, cooked elderberry, white-hearted cherry; *almost free from vitamin*: whortleberry, cranberry (cooked), stored apple, pear, quince (raw and cooked), grape, dried fruits (*e.g.* date, currant, fig, etc.). The minimum weight of fruit to prevent symptoms of scurvy in man is given as 50 times that required by guinea-pigs.

J. G.

**Effects of Overdosage of Vitamin D. II.** R. F. Light, G. E. Miller and C. N. Frey. (*J. Biol. Chem.*, 1931, 92, 47-52.)—In a study of the effect of low and high vitamin D overdosage on the reproduction of white rats the authors had available a number of animals of the third and fourth generation, some of the progenitors of which had received 40 units and others 2500 units of vitamin D as supplements to the basal diets. It was decided to use some of these animals in a study of the ash content of bones and of organs which had been found to be particularly susceptible to calcification, namely, heart and kidneys. The animals were examined in four groups. The results show that moderate overdosage (40 units) of vitamin D daily has no effect on the mineral metabolism of white rats when continued through the third and fourth generations. No pathological calcification occurs, and the ash of the bones is normal. Animals receiving a moderate overdosage of vitamin D for a long period of time are more susceptible to excessive overdosage than normal animals. A large overdosage (2500 units) of vitamin D just insufficient to produce toxic symptoms in the first and second generations, given for a long

period of time, does produce striking pathological changes in the third and fourth generations. These changes are (a) decalcification of the bones, (b) severe calcification of the kidneys, and (c) certain pellagra-like symptoms, namely, scabby conditions of the feet, nose and forequarters.

P. H. P.

## Organic Analysis.

**Detection and Separation of Hydrocarbons with Branched Chains from Natural or Artificial Mixtures of Hydrocarbons.** A. Schaarschmidt. (*Chem. Ztg.*, 1931, 55, 424.)—The olefines and aromatic compounds present in hydrocarbon mixtures are easily determined and separated, and in the residual mixture of saturated aliphatic and alicyclic hydrocarbons, the latter may be determined with moderate accuracy, although not isolated, by means of the aniline point, in accordance with Tizard and Marshall's method (*ANALYST*, 1921, 46, 155). It is now found that, with the more simple mixtures obtained from natural or synthetic hydrocarbons, treatment with a metallic halide with a mobile halogen atom, such as antimony pentachloride, renders it possible to detect and separate saturated aliphatic and alicyclic hydrocarbons containing trebly-linked carbon atoms; the complex compounds formed are insoluble in excess of the hydrocarbon mixture, and form characteristic greenish-blue or violet products with water or alcohol. Normal hydrocarbons and those containing quaternary-linked carbon atoms remain behind. This method has been applied to different fractions obtained from synthetic and natural hydrocarbons, it being found, for example, that benzine fractions with b.pt. about 100° C. consist mostly of aliphatic and alicyclic hydrocarbons with branched chains.

T. H. P.

**New Acid in the Oil of *Conepia grandiflora*, Benth.** F. Wilborn. (*Chem. Ztg.*, 1931, 55, 434.)—An oil, with  $n_D^{25}$  1.5094, has been extracted from the seeds of *Conepia grandiflora* (*Rosaceae*); it has similar properties to Chinese wood oil, and contains a highly unsaturated acid, probably isomeric with elaeostearic acid, having neutralisation value, 200.3; m.pt., 94° C.;  $n_D^{25}$ , 1.4865; and molecular weight (Rast), 267.

J. G.

**Identification of Mesaconic Acid.** H. H. Mottern and G. L. Keenan. (*J. Amer. Chem. Soc.*, 1931, 53, 2347–2349.)—Mesaconic acid (a natural constituent of plants), its hydrazide, and *p*-nitro-benzyl mesaconate are crystalline bodies readily studied by the optical immersion method. The pure acid prepared by the method of Fittig (*Annalen*, 1877, 188, 73) had a melting point of 204.5° C. (corr.). When sublimed it yields colourless rod-like forms in laminated fibrous masses. With crossed nicols the rods show parallel extinction, and the elongation is + or –; the double refraction is very strong, and the index of refraction (immersion method) is less than  $n = 1.445$ . The acid is soluble in oily mixtures; the maximum refractive index was 1.740 (methylene iodide), but this was infrequent; the most significant refractive index is shown when the elongated crystals are oriented with their long axis parallel to the vibration plane of the lower nicol, when the rods

frequently match a liquid with  $n = 1.690$  (conveniently monochloronaphthalene and methylene iodide). Diethyl mesaconate was prepared by heating the acid under reflux for 18 hours with 300 c.c. of absolute alcohol, dissolving the ester in ether, and washing with dilute sodium hydroxide solution, drying with anhydrous sodium sulphate and distilling off the ether under reduced pressure. The diethyl mesaconate boiled at  $93-95^{\circ}\text{C}$ ., under 10 mm. pressure. Mesaconic hydrazide was made from the ester by adding 1 c.c. of 42 per cent. hydrazine hydrate in water to a solution of 0.7 gm. of the ester in 5 c.c. of absolute alcohol and, after standing, crystallising the hydrazide; the purified crystals melted at  $217-218^{\circ}\text{C}$ . with decomposition. The *p*-nitrobenzyl mesaconate prepared by the method of Reid (*J. Amer. Chem. Soc.*, 1917, 39, 124) had a melting point of  $134^{\circ}\text{C}$ . Optical crystallographic data are given for both these derivatives. D. G. H.

**Iodine Mercerisation Test.** W. F. A. Ermen. (*J. Soc. Dyers and Col.*, 1931, 47, 161.)—Mercerised and unmercerised samples of cotton fabrics are immersed in a solution of six grms. of iodine in 100 c.c. of potassium iodide solution. They are washed until the unmercerised sample is colourless and are then dropped into a boiling solution of Indigosol Black 1B. The mercerised sample is deeply dyed, the unmercerised emerging colourless. The samples are now washed, first in cold water, and finally in boiling soap solution. The Indigosol requires to be freshly prepared every time. In the case of an unknown sample, starch should be tested for, and, if present, removed either by boiling with dilute acid or by treatment with malt extract. Comparative tests must be made with cloths of similar substance. R. F. I.

## Inorganic Analysis.

**Volumetric Determination of Carbon Monoxide by means of a Suspension of Iodine Pentoxide in Fuming Sulphuric Acid.** H. A. J. Pieters. (*Chem. Weekblad*, 1931, 28, 335-337.)—The gas is measured in a water-jacketed (35 c.c.) burette, and carbon dioxide then removed in an absorption vessel containing 40 per cent. potassium hydroxide solution. The residual gas is passed into a 10 per cent. suspension of iodine pentoxide in fuming sulphuric acid (10 per cent.  $\text{SO}_3$ ), contained in a double absorption pipette, the outlet from which leads through a wash-bottle containing sulphuric acid to the air. The gas is then returned to the absorption vessel, when the carbon dioxide formed by oxidation of the carbon monoxide is absorbed in the alkali, the absorption being measured manometrically. The reaction period varies with the carbon monoxide content (10, 15 and 20 minutes for less than 0.06, 2.8 and 5.2 per cent. by volume, respectively), but the end of the reaction is indicated when no further absorption of carbon dioxide occurs. An accuracy of 0.02 per cent. was obtained with mixtures of known composition and as compared with results obtained by the ammoniacal cuprous chloride reagent, and the method is unaffected by the presence of hydrogen or methane, both of which, however, are slowly oxidised by a reagent containing 25 per cent.  $\text{SO}_3$ . J. G.

**Volumetric Determination of Copper based on Spacu's Reaction.**

L. Cuny. (*J. Pharm. Chim.*, 1931, 13, 513-518.) J. Golse (*ANALYST*, 1931, 272) published criticisms of Cuny's original method (*J. Pharm. Chim.*, 1924, 30, 240), and proposed a modified process. Cuny now agrees with practically all Golse's findings, and states that the grossest fault of his original method lay in the use of the empirical and inaccurate permanganate titration method for determining the excess of thiocyanate remaining in the solution after the precipitation of the copper as copper pyridine thiocyanate.

S. G. C.

**Determination of Lead and Copper in Bordeaux and Lead Arsenate Mixtures.** J. C. Bubb. (*J. Assoc. Off. Agric. Chem.*, 1931, 24, 260-263.)—

As an equally accurate alternative to the more lengthy official method of the A.O.A.C. for the determination of lead oxide and copper, the following method is proposed: A mixture of 1 grm. of the sample with 50 c.c. of "acetic acid solution (1 + 2)," contained in a 250 c.c. beaker, is heated on a steam-bath for 5 to 10 minutes; 0.5 grm. of calcium arsenate is added to convert any tri-lead arsenate,  $Pb_3(AsO_4)_2$ , or other lead compound, into the acid arsenate,  $PbHAsO_4$ , followed by nitric acid, drop by drop, with stirring, until any blue coloration in the insoluble residue clears up and the residue of lead arsenate is white (up to 1.5 c.c. of nitric acid is required, and the amount used is noted). The mixture is cooled in a water-bath, ammonia is added in amount exactly equivalent to the nitric acid added; the mixture is kept at room temperature for 20 minutes, and then filtered (No. 44 Whatman paper), the insoluble residue being washed with small portions of hot water. The residue is dissolved by heating in dilute nitric acid (20 per cent.) the lead determined in the solution by the official chromate method of the A.O.A.C. (*Methods of Analysis*, 1925, 58), and the result calculated to  $PbO$ . The copper in the filtrate is determined by adding potassium iodide and titrating with thiosulphate in the usual manner.

S. G. C.

**Study of Travers' Method for the Determination of Fluorine with Reference to Insecticides.** C. M. Smith, E. H. Hamilton, and J. J. C. Graham. (*J. Assoc. Off. Agric. Chem.*, 1931, 24, 253-260.)—The following modified form of Travers' method is proposed for the analysis of insecticides containing fluorides: To 0.5 grm. of the sample, contained in a small beaker, from 20 to 25 c.c. of water, 0.3 grm. of finely divided *precipitated* silica and a few drops of methyl orange indicator are added. Concentrated hydrochloric acid is added, drop by drop, to the mixture until the indicator changes to pink, followed by 2 c.c. of the acid in excess; the liquid is boiled for 1 minute and then cooled. Four grms. of solid potassium chloride are dissolved in the liquid, 25 c.c. of ethyl alcohol (96 per cent.) are added, and the whole kept for 1 hour with frequent stirring. The solution is filtered through a Gooch crucible with an asbestos filtering-pad (fritted glass will not do), and the precipitate washed with an alcoholic solution of potassium chloride [60 grms. of potassium chloride dissolved in 400 c.c. of water with 400 c.c. of ethyl alcohol (96 per cent.) added; this solution should be made neutral to phenolphthalein] until the washings are practically neutral to

phenolphthalein (three or four washings are usually sufficient). The crucible and contents are placed in a 400 c.c. beaker together with 100 c.c. of freshly boiled water and 1 or 2 c.c. of phenolphthalein solution (1 per cent.); the liquid is heated and titrated with standard sodium hydroxide solution (0.2 *N*; freedom from carbonate specified; 1 c.c. = 0.0057 grm. of fluorine), the titration being finished with the liquid actively boiling. The method is stated to be satisfactory for, *e.g.* chicken-lice and cockroach powders, which consist of commercial sodium fluoride containing as impurities sodium carbonate, sodium sulphate, sodium bifluoride, and sodium silicofluoride. Most fluoride insecticides are mixtures of fluorine compounds either with other insecticidal materials or with diluents. The effect on the method, of a number of different substances was tried. The following (about equal in amount to the fluoride taken) gave no interference: lime, sulphur, starch, flour, tobacco, talc, *p*-dichlorobenzene, naphthalene, cresols, coal-tar neutral oils, Paris green and calcium arsenate. Pyrethrum and lead arsenate caused a tendency to high results. Slightly low results were obtained with added kaolin, diatomaceous earth and ferric chloride. The process fails in presence of soluble aluminium or boron compounds, very low results being obtained. S. G. C.

**Electrometric Titration of Uranium by means of Ceric Sulphate.**

D. T. Ewing and M. Wilson. (*J. Amer. Chem. Soc.*, 1931, 53, 2105-2110.)—The hot sulphate solutions in 2 per cent. sulphuric acid were passed through a Jones reductor, collected in an atmosphere of nitrogen, and titrated electrometrically with ceric sulphate. Two distinct end-points were obtained: (1) Trivalent to quadrivalent, and (2) quadrivalent to hexavalent uranium. If ferrous salt is also present, its oxidation to ferric salt follows the complete oxidation of the uranium, and a third end-point is observed. W. R. S.

**Colorimetric Determination of Sodium.** R. A. McCance and H. L.

Shipp. (*Biochem. J.*, 1931, 25, 449-456.)—A method is described by which 0.02-0.8 mgrm. of sodium may be directly determined; in the absence of phosphates the range is 0.01-0.4 mgrm. of sodium. The free acids and phosphates are removed with zinc acetate and hydroxide in 50 per cent. alcohol, then the sodium is precipitated as sodium uranyl zinc acetate, and the uranium in the precipitate is determined colorimetrically with potassium ferrocyanide. The method is applicable to neutral or acid solutions. Calcium, magnesium and iron do not interfere; phosphates interfere, but are removed. Sodium may be determined directly in the presence of 30 times its weight of potassium. The following reagents are necessary:—(1) *Alcoholic zinc acetate with zinc hydroxide.*—A slight excess of ammonia (sp. gr. 0.880) is added to a hot strong solution of A.R. zinc sulphate; this is filtered on a Buchner funnel, washed with hot water, and sucked dry. The zinc hydroxide paste formed is added in small amounts at a time, until in slight excess, to 12.5 c.c. of glacial acetic acid, filtered, washed, the combined filtrate and washings made up to 100 c.c., then 3 c.c. of ammonia and 300 c.c. of 95 per cent. alcohol are added. (2) *Alcoholic uranyl zinc acetate reagent.*—(a) In 50 c.c. of

boiling water containing 2.0 c.c. of glacial acetic acid 10 grms. of uranyl acetate are dissolved. (b) In 50 c.c. of boiling water containing 1 c.c. of glacial acetic acid 30 grms. of zinc acetate are dissolved. Both solutions are mixed while boiling, brought to boiling point, left overnight, then filtered. The filtrate is mixed with an equal volume of absolute alcohol, left for 48 hours at 0° C., and filtered at 0° C. The reagent is stable at room temperature. (3) *Ninety-five per cent. alcohol saturated with the triple acetate*.—Uranyl zinc acetate reagent is added to sodium chloride dissolved in 50 per cent. alcohol, filtered or centrifuged, and the precipitate of sodium uranyl zinc acetate washed with 95 per cent. alcohol. The precipitate is suspended in 95 per cent. alcohol and left to settle in the ice-chest. The fluid is used for washing the precipitate. It must be filtered before use if not absolutely clear. (4) *Twenty per cent. potassium ferrocyanide*. (5) *Standard sodium chloride*.—The stock solution contains 1 grm. of pure dry sodium chloride in 100 c.c. of water. For use, 2 c.c. are diluted to 100 c.c.; thus 1 c.c. contains 0.2 mgrm. of sodium chloride (0.0786 mgrm. of sodium). (6) *Standard triple acetate*.—To 10 c.c. of 1 per cent. sodium chloride solution, 80 c.c. of water, 100 c.c. of alcohol and 100–120 c.c. of reagent (2) are added, and left for 1 hour; the precipitate is collected, washed with ice-cold 95 per cent. alcohol, dissolved in water, and made up to 1000 c.c. to form the stock solution. A portion is diluted accurately 1 : 5. To 5 c.c. of this solution, diluted with water in a 25 c.c. flask, 1 drop of glacial acetic acid and 0.5 c.c. of reagent (4) are added, and the whole made up to 25 c.c. The resulting colour is close to that obtained from 0.2 mgrm. of sodium chloride (0.0786 mgrm. of sodium) in 2 c.c. of water submitted to all stages of the method and made up to 25 c.c. This triple acetate solution must be accurately standardised against sodium solutions. Four samples of exactly 0.2 mgrm. of sodium chloride in 2 c.c. of water are subjected to all stages of the determination, and the precipitates transferred to 25 c.c. flasks. In 2 other flasks 5 c.c. of the dilute standard are placed, and water is added to 18–20 c.c. The uranium colour is developed in all six flasks. Both standards are matched against each of the quadruplicate flasks, setting the latter at 20 mm.; the mean is taken. If this should be 23 mm., it is best always to set the standard at 23 mm. in the colorimeter. (This is equivalent to the colour obtained by determination from 0.0786 mgrm. of sodium, the precipitate being made up to 25 c.c., and the colorimeter set at 20 mm.); or, if preferred, the stock solution may be diluted so that 5 or 10 c.c. of the weak standard diluted to 25 c.c. gives exactly the same colour intensity as that obtained from 0.0786 mgrms. of sodium. *Procedure*.—An amount of the unknown solution containing 0.04 to 0.16 mgrm. of sodium is placed in a centrifuge tube, diluted to 2 c.c. with water, 4 c.c. of reagent (1) added, stirred, and covered with a rubber cap (10 c.c. vaccine cap), then left 2–3 hours at room temperature, and at 0° C. overnight. It is centrifuged while cold, and 3 c.c. of the supernatant liquid are placed in another centrifuge tube, 4 c.c. of reagent (2) are added, and the whole is stirred with a glass rod until the precipitate begins to appear. It is covered with a rubber cap and left for 1 hour at 0° C., centrifuged, the liquid poured off, and the tube drained by inverting on filter-paper. The mouth of the



tube is wiped, and the inside and contents are washed once with 5 c.c. of the ice-cold alcohol saturated with the precipitate. The precipitate should be stirred up, centrifuged, and again drained. It is dissolved in water, and transferred to a 25 c.c. volumetric flask (unless too bulky, when a larger flask is necessary). For the standard either 1 c.c. of the dilute sodium chloride solution is treated in the same way as the unknown and transferred to a 25 c.c. flask, or 5 c.c. of the dilute standard triple acetate solution are taken in a 25 c.c. flask. To both standard and unknown, 1 drop of glacial acetic acid and 0.5 c.c. of reagent (4) are added, the solutions are made up to the mark with water, left for 3 minutes, and matched. *Calculation.* (a) *With standard sodium chloride solution.*—Sodium, mgrm. per 100 c.c.

$$= \frac{20 \text{ (standard colorimeter reading)}}{\text{Reading of unknown}} \times 0.0786 \times \frac{100}{\text{Volume of unknown taken}}.$$

(b) *With standardised triple acetate solution.*—Suppose the colorimeter set at 23 mm. is equivalent to 0.2 mgrm. of sodium chloride submitted to all stages of the determination made up to 25 c.c. and set in the colorimeter at 20 mm. Then, sodium, mgrm. per 100 c.c.

$$\frac{20}{\text{Unknown}} \times 0.0786 \times \frac{100}{\text{Volume of unknown taken}}$$

A communication will shortly be made on the application of the method to blood and serum without incineration. P. H. P.

**Determination of Traces of Chloride in Bromides.** I. E. Orlow. (*Z. anal. Chem.*, 1931, **84**, 185–189.)—A solution of 5 grms. of bromide is treated with 50 c.c. of 20 per cent. sulphuric acid and 3 grms. of freshly-precipitated dried manganese peroxide. The mixture is diluted to 150 c.c. and boiled for 30 minutes; if the volume is thereby reduced to less than 50 c.c., hot distilled water is to be added. The liquid should no longer smell of bromine, otherwise it is boiled for another 10 to 15 minutes with a small addition of the dilute sulphuric acid. The cold liquid is made up to 200 c.c., and 100 c.c. titrated with silver solution. The process, which requires half an hour, can also be used for the determination of chloride in potassium iodide. W. R. S.

**Sensitive Test for Iodine.** C. V. King and M. B. Jacobs. (*J. Amer. Chem. Soc.*, 1931, **53**, 1704–1714.)—In an investigation into the kinetics of the reaction between iodide and persulphate in highly dilute solution, which forms the subject of this paper, use was made of a balanced photo-cell connected with a sensitive galvanometer to detect the time of first appearance of iodine in the solution. The test is stated to be much more sensitive than the reaction with starch or with basic lanthanum or praseodymium acetate. A diagram and particulars of the photo-cell circuit are given. W. R. S.

**Internal Indicators for Bromate Titrations.** G. F. Smith and H. H. Bliss. (*J. Amer. Chem. Soc.*, 1931, **53**, 2091–2096.)—A number of dyes were studied with reference to their suitability as indicators for the bromate titration

of, e.g. arsenic and antimony, in which methyl orange or indigosulphonic acid are usually employed. Several monoazo- and triphenylmethane dyes proved satisfactory; fuchsine was found to be preferable to methyl orange, as the titration could be made at room temperature and at lower acidities. Whilst the action of bromate on methyl orange requires 3 *N* acid at a temperature not below 60° C., the indicators tested were bleached at *N* acidity, the required temperature varying between 25° and 85° C.

W. R. S.

**Volumetric Determination of Fluorine by means of Cerous Nitrate.** G. Batchelder and V. W. Meloche. (*J. Amer. Chem. Soc.*, 1931, 53, 2131–2136.)—Sodium fluoride solutions were treated with a known excess of cerous nitrate, and the non-precipitated cerium salt titrated with permanganate. Removal of the mixed precipitate by filtration in the neighbourhood of the end-point was found to be necessary, making the method rather cumbersome. The direct titration of neutral fluoride solutions with cerous nitrate in presence of an adsorption indicator was found to be convenient, amphotomagenta (0.02 grm. per litre) changing from deep blue to a less intense purple at the equivalence point (the dye is diazotised *p*-aminoethylacetanilide coupled with 1,8-dihydroxynaphthalene-3,6-disulphonic acid). Kurtenacker and Jurenka's procedure (*Z. anal. Chem.*, 1930, 82, 210), which consists in carrying out the same titration in presence of methyl red, was also tried, and found to give satisfactory results. No end-points could be obtained in presence of sulphate ion.

W. R. S.

**Iodimetric Thiocyanate Titration.** H. A. Pagel and H. J. Koch. (*J. Amer. Chem. Soc.*, 1931, 53, 1774–1777.)—The neutralised thiocyanate solution is treated with 1 grm. of ammonium sulphate, nitrate, or chloride dissolved in 20 c.c. of *N* ammonia, and an excess of 5 c.c. of standard iodine solution, and left for 5 minutes. It is then acidified with 6 *N* hydrochloric acid (5 c.c. excess) and immediately titrated with standard thiosulphate solution, which gives the excess of iodine over  $\text{HCNS} + 3\text{I}_2 + 4\text{H}_2\text{O} = \text{H}_2\text{SO}_4 + \text{HCN} + 6\text{HI}$ . Nickel, cobalt, and manganese cause erratic results; precipitated aluminium hydroxide, zinc, magnesium, and borax do not interfere.

W. R. S.

## Microchemical.

**Contributions to the Micro-Dumas Method.** O. R. Trautz. (*Mikrochem.*, 1931, 9, 300–312.)—Sources of error in the micro-Dumas determination of nitrogen are investigated. (1) *Dissociation in the heated combustion tube.*—Dissociation of carbon dioxide into carbon monoxide and oxygen is shown by enlargement of the micro-bubbles, since these gases are not absorbed in 50 per cent. potash solution. In the conditions of the experiment no enlargement of micro-bubbles is observed, even on prolonged heating of the tube, with a rate of flow of carbon dioxide of 22 c.c. per minute (7 bubbles per second). Copper oxide wire

of 0.5 mm. diameter is used rather than coarser wire, as with smaller spaces between the wires there is a longer time of contact between the gases and the copper oxide. The copper oxide of the temporary filling must be oxidised after use by igniting for half-an-hour and cooling in air for a day or more. (2) *Purity of the carbon dioxide*.—The micro-bubbles consist chiefly of air which comes from that dissolved in the hydrochloric acid of the Kipp generator. It is found that 1000 bubbles of diameter 0.2 mm., or 40 to 50 c.c. of carbon dioxide, which is the volume used to sweep out the apparatus, cause an air error of 4 c.mm., which has not previously been taken into account. Purer carbon dioxide, with a constant air content and giving micro bubbles of 0.1 mm. diameter, is obtained by using two Kipp generators in series (Niederl, Trautz and Saschek, *ANALYST*, 1930, **55**, 771), or by evacuating the upper bulb of the Kipp generator, using a Hein pump (*Z. angew. Chem.*, 1927, 864). The air content should be determined, and the correction of 1–6 c.mm. per 100 c.c. of carbon dioxide applied. When the air content is greater than 4 c.mm. per 100 c.c. it is best to insert a gasometer between the generator and combustion tube so that a standard amount of carbon dioxide can be used for each analysis and the correction applied more accurately. (3) *Adsorption error in the combustion tube*.—The permanent filling is always left under pressure of carbon dioxide, and any air which enters it during the replacement of the temporary filling is removed by a stream of carbon dioxide in the cold, but the adsorbed air is not completely removed from the temporary filling in the cold. Pregl's method (*Quantitative Organic Micro-analysis*, 2nd Ed., 1930) is to heat the tube, but there is danger of loss in the analysis of volatile substances. Therefore, the tube is swept out in the cold for 5 minutes with carbon dioxide (about 150 c.c.) and a correction is made for the adsorption error of the temporary filling. This has been found to be 6 c.mm. (4) *Nitrometer errors*.—The adherence of bubbles to the mercury-potash surface is avoided by adding a few drops of mercury which have been shaken with ether, and, after removing the ether, washed with water and potash solution; both the mercury and the slight sludge formed are added to the mercury in the nitrometer. The wall error, due to the moistening of the sides of the nitrometer with potash has been re-determined and found to be 0.5 per cent., as compared with the value 1.7 per cent. used by Pregl. The correction for the vapour pressure is 0.3 per cent. of the volume of nitrogen. The amount of nitrogen absorbed in the potash solution has been found to be negligible, showing the possibility of determining even smaller amounts of nitrogen. (5) *Rubber connections*.—It is found that a piece of best quality rubber, 7 mm. long, 2 mm. inside diameter, and 1 mm. wall thickness in the pressure conditions of the experiment, allows 1 c.mm. of air per hour to diffuse through. Therefore the error is negligible when the rubber connections are good, but they must be tested frequently. It is better to use ground-glass joints. When a known amount of carbon dioxide is used for the sweeping out, and the corrections described are applied, the analysis can be carried out at a rate of 4 bubbles per second, so that the determination can be completed in 15 minutes, and results are obtained which show no difference from those at the slower rate of analysis of 1 bubble per second.

J. W. B.

**Micro-Determination of Copper by means of Salicyl-aldoxime.** W. Reif. (*Mikrochem.*, 1931, 9, 424-429.)—Salicyl-aldoxime, a reagent first used by Ephraim (*Ber.*, 1930, 63, 1928) is used for the micro-determination of copper. *Method.*—From 2 to 5 c.c. of the test solution are treated, drop by drop, with dilute ammonia, until the blue colour is clearly visible, when ammonium acetate is added until the blue colour disappears again. The copper is then precipitated with a freshly prepared alcoholic solution of salicyl-aldoxime. The mixture is shaken until the green-white precipitate agglomerates, and further salicyl-aldoxime is then added until excess is present. After standing for 10 to 20 minutes the precipitate is filtered by means of a Pregl micro-filter tube and washed alternately with cold water and alcohol, and finally with alcohol, and dried in a Pregl drying block at 105° C., cooled and weighed. The factor is 0.1895, and the log. factor is 1.27761. On amounts of copper from 0.7 to 1.3 mgrm. the error is about 0.5 per cent. The determination can be carried out with a similar accuracy in the presence of ammonium salts; the error is only 1 per cent. in the presence of 10,000 times the weight of ammonium chloride. In the presence of iron about 50 to 100 mgrms. of tartaric acid are added to the test solution before the first treatment with ammonia, to give a stable tartrate complex with the iron, which then does not affect the accuracy of the determination. For the determination of nickel and copper in the same solution, the test solution is treated with about 50 mgrms. of tartaric acid, and then with ammonia until the blue colour appears. The nickel is then precipitated with a 1 per cent. alcoholic solution of dimethylglyoxime, and the tube is heated over a water bath at 70° to 80° C. for  $\frac{1}{2}$  hour until the red precipitate coagulates. The solution is tested to see if the precipitation is complete; and after standing, the mixture is filtered through a Pregl filter tube, and the precipitate dried at 110° C. and weighed. The filtrate is treated with ammonium acetate until the blue colour disappears, when the copper is precipitated as before.

J. W. B.

## Physical Methods, Apparatus, etc.

**Transference of Small Quantities of Liquids.** G. Owen. (*J. Soc. Chem. Ind.*, 1931, 50, 189T-190T.)—Small quantities of liquids ( $\frac{1}{2}$ –1 c.c.) which have been sealed up in ampoules may be manipulated with ease if a siphon of finely drawn-out and bent glass tubing of about 0.2 mm. bore is inserted to the bottom of the ampoule. The liquid automatically rises in the tube by capillarity and flows out, drop by drop.

P. H. P.

**Apparatus for Recording the Ultra-Violet Light of the Sky.** J. R. Ashworth. (*Nature*, 1931, 127, 893.)—Hill's method of measuring ultra-violet light by recording the fading of an acetone solution of methylene blue is simple and satisfactory, except that on short winter days the fading produced is too slight to be observable by this method. An alternative procedure, which has been under test during the last year or two in the neighbourhood of Rochdale, involves the use of

photographic printing paper and has given monthly average readings agreeing well with those furnished by the methylene blue method. A strip ( $3 \times 1$  inch) of ultra-violet glass which is opaque to visible light but transparent to a band of rays with the wave-length range 3400–3700 A.U., is fitted into a slot ( $3 \times 1$  inch) in the lid of a shallow metal box. A strip of photographic paper, on the bottom of the inside of the box, is covered with a stepped "wedge," constructed of layers of thin tissue paper of fine quality arranged to provide ten different thicknesses through which the light may pass.

The closed box is exposed to the light of the sky, and at the end of the day the photographic paper is examined to ascertain the greatest number of layers of the tissue paper which the light has penetrated. According to theory this number is the logarithm of the intensity of the light, and, the transmission factor of the paper being known, an arbitrary scale of light values may be constructed. This method gives readings on all winter days except when fog obscures light of all kinds. As summer approaches and the light becomes stronger, the wedge scale may need extension; a further suitable number of layers of the tissue paper may be superimposed on the wedge without detriment to the accuracy of the readings. If the photographic paper is wrapped on a rotating drum, a continuous record is obtainable.

T. H. P.

**Filters made of Porous Hard Rubber.** E. Vossen. (*Chem. Ztg.*, 1931, 55, 454.)—A filtering medium of porous hard rubber containing 32.08 per cent. of sulphur is completely resistant to the action of hydrofluoric acid, sulphurous acid, phosphorous acid, phosphoric acid, alkalis and solutions of salts, and also to sulphuric acid (less than 70 per cent. and below 70° C.) and hydrochloric acid (less than 20 per cent. and below 20° C.). The range of temperature within which it can be used is, in general, from  $-10^{\circ}$  C. to  $110^{\circ}$  C. The manufacture of these filters is, in outline, as follows: Rubber, sulphur, and vulcanising fluxes are kneaded to a homogeneous mass which is then pulled out into sheets and vulcanised just sufficiently to allow of the product being ground into a powder. The pulverised material is separated, by sieving, into grades of different fineness which yield products of different degrees of porosity according to the fineness. The grades are separately vulcanised under pressure in iron moulds of the required shape; the porous product is finally treated with silica-gel in order to remove carbon disulphide which is present in the pores. These porous rubber filters find their main application as substitutes for filter cloth, etc., in the form of plates, rings and tubes for filter presses and suction filters. It is stated that it is not known at present whether it will be possible to reduce the pore-size sufficiently to render the porous rubber suitable for colloid or bacteria filters.

S. G. C.

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## References to Scientific Articles not Abstracted.

**PRESERVATION OF LEATHER BOOKBINDINGS.** By R. W. FREY and F. P. VEITCH.  
*Leaflet No. 69. U.S.A. Dept. of Agriculture.*

Rotting of Leather Bookbindings—Dressings for Bindings—Application of Dressings—Treating Vellum Bindings—Lacquering Powdery Bindings.

**NEW ALKALOIDS DISCOVERED, 1920–1929 INCLUSIVE.** By J. F. COUCH. *Amer. J. Pharm.*, 1931, 103, 242–251 (May).

An Alphabetical Summary of New Alkaloids, with formulae and melting points. Bibliography containing 89 references.

**THE PATHOLOGY OF SOME INDUSTRIAL POISONS.** By A. J. AMOR. *Chem. and Ind.*, 1931, 50, 475–476 (June 5).

Carbon Monoxide: Action on Haemoglobin and on Brain Cells—Arsine: Haemolysis of Red Blood Corpuscles—Effect on Stomach and Kidneys—Lead: Anaemia of Lead Poisoning—Lead Paralysis—Lead Colic—Lead Encephalopathy—Effect on the Arteries—Effect on the Kidneys.

## Reviews.

**HANDBOOK OF CHEMICAL MICROSCOPY.** Vol. II. By E. M. CHAMOT, Ph.D., and C. W. MASON, Ph.D. 2nd Edition. Pp. ix+411, with 181 Figures. London: Chapman and Hall. 1931. Price 22s. 6d.

The opinion has frequently been expressed that the attraction of chemical microscopy to those who employ it lies in the manipulations involved much more than in the results attained. That this view is erroneous is well illustrated by the present work in conjunction with Volume I previously reviewed (*ANALYST*, 1930, 55, 470), and in the ever-increasing applications of microscopical analysis in many branches of science. Those chemists who have adopted these methods soon realise that for economy of materials, reagents and time, as well as certainty of the results obtained, the usual macroscopic methods are left far behind, and the brief practice necessary to acquire the special technique used is very soon justified.

This volume comprises chapters dealing with the technique of manipulating small amounts of material, methods of using reagents, reactions of the elements arranged according to the eight groups of the periodic system, detection of inorganic anions together with acetic, oxalic and tartaric acids, special reagents, qualitative micro-analysis of mixtures including alloys, and an accurate and well-arranged index. The general plan adopted in the descriptions given is roughly as follows: (a) discussion of the reactions of each member of a group,

(b) detection of one or more group metals present in the unknown substance, (c) behaviour of a group reagent with elements of other groups, (d) separation of the metals present when necessary, and (e) identification of the separated metals by appropriate reactions. The description of the methods and of the results obtained is particularly lucid, although in certain cases necessarily involved, but all redundant matter has been carefully excluded, and it will be found that the practical application of many of the reactions occupies much less time than their perusal.

The reactions described have been well selected, and are dependable, with, perhaps, the exception of those involving the use of potassium xanthate, but it appears to the reviewer that insufficient reference is made to the use of the flame test in conjunction with a small hand spectroscope and to the value of carrying out simultaneously on the same microscope slide three or four tests with the same reagent, using different concentrations of the solution under examination.

The numerous illustrations, of which a large proportion are photomicrographs depicting crystalline precipitates obtained in various reactions, are excellent, and add considerably to the value of the work, while the freedom from typographical and other errors, the legibility of the text, and the general style of the book make it a worthy successor to the previous volume. This work is a valuable addition to the literature of the student and the analytical chemist, and it is to be hoped that the authors will provide a third volume on similar lines dealing with the chemical microscopy of the organic acids and radicals.

T. J. WARD.

**Erratum.**—*Bell's Sale of Food and Drugs Acts.* The case cited in the review in the June issue (p. 421), should be *Lémy v. Watson* (not "*Lamy*").



# THE ANALYST

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## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

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### Deaths.

WITH deep regret we record the deaths of the following members:

GEORGE CRAIG, on May 5th.

HENRY DROOP RICHMOND, on August 26th.

An obituary of Mr. Richmond will be published in a subsequent issue.

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### Carbon Monoxide Poisoning:

#### Its Detection, and the Determination of Percentage Saturation in Blood, by Means of the Hartridge Reversion Spectroscope.

By ROBERT C. FREDERICK, A.I.C.

**INTRODUCTION.**—So much has been heard in recent years of carbon monoxide poisoning that there is a tendency to regard casualties from this cause as being peculiar to the age. Though cases are now apparently more numerous, the number has always been large, and Lewin<sup>1</sup> goes so far as to state that carbon monoxide is now, and has been since the first discovery of fire, the most widespread poison connected with human life and activity. The vehicle in which the poison is conveyed has, however, certainly changed with the times.

In the literature nearly a century ago there is discussion regarding the many fatalities occurring in France (a large proportion of which were suicidal) due to the use of braziers. French writers of fiction in their works frequently introduced



situations where death took place by this means, and one, Zola, himself met his death accidentally in this way. The use of fire fumes was a method of suicide employed by the Romans.

At the present time, in this country, the most common cause of carbon monoxide poisoning is the public gas supply, and history is repeating itself, for the majority of these cases are suicidal. The extremely poisonous character of the gas is due to the carbon monoxide content being increased by admixture with water-gas and similar products. The Report of the Departmental Committee appointed to consider the increase in the number of deaths ascribed to poisoning by coal gas supplied for domestic purposes<sup>2</sup> reveals that such as were suicidal increased from 193 in 1918 to 1191 in 1928; the figures for those considered officially as due to accident were 102 and 168, respectively. Apart from the question of gas escape, poisoning may occur when the gas is burning if the flame is striking a cold conducting material; hence the menace of geysers and other types of hot water heaters when fitted without adequate flues.

The advent of the petrol engine has brought in its train numerous cases of poisoning, not all accidental, due to the carbon monoxide in the exhaust gases. A certain number of cases of poisoning by carbon monoxide have occurred with men seeking warmth and falling asleep by the slow-burning heaps of town and ironworks refuse. Poisoning by carbon monoxide is an occupational hazard in many industries, amongst which are water gas, producer gas, and coal gas manufacture; blast furnace and coke oven operation; lime and charcoal burning; and coal mining.

The physiological response of the individual to various concentrations of carbon monoxide has been studied by Henderson, Haggard, Teague, Prince, and Wunderlich,<sup>3</sup> and their findings have been summarised by the two first-named in the following tables<sup>4\*</sup> :—

Concentration.	Carbon monoxide. Per Cent.
Allowable for an exposure of several hours .. .. .	0.01
Can be inhaled for one hour without appreciable effect .. .. .	0.04-0.05
Causing a just appreciable effect after one hour's exposure .. .. .	0.06-0.07
Causing unpleasant but not dangerous symptoms after one hour's exposure .. .. .	0.1-0.12
Dangerous for exposure of one hour .. .. .	0.15-0.20
Fatal in exposure of less than one hour .. .. .	0.4 and above

The poisonous effect of carbon monoxide is exercised through the blood owing to the great affinity of the gas for haemoglobin (some 300 times that of oxygen), which results in the formation of carboxyhaemoglobin. The carboxyhaemoglobin is entirely incapable of carrying oxygen to the tissues, and according to the amount of haemoglobin thus inactivated (or percentage saturation) so the individual exhibits illness of proportionate severity; if the percentage saturation becomes sufficiently high, death ensues. The average physiological effects

\* Modified to show figures in terms of percentage, instead of parts per million.

caused by varying percentage saturations of the blood with carbon monoxide have been succinctly set out by Henderson and Haggard in the following table\*—

Haemoglobin in combination with carbon monoxide. Per Cent.	Physiological effect.
10	No appreciable effect except shortness of breath on vigorous muscular exertion.
20	No appreciable effect in most cases except short wind even on moderate exertion; slight headache in some cases.
30	Decided headache; irritation; ready fatigue; disturbance of judgment.
40-50	Headache, confusion, collapse and fainting on exertion.
60-70	Unconsciousness; respiratory failure and death if exposure is long continued.
80	Rapidly fatal.
Over 80	Immediately fatal.

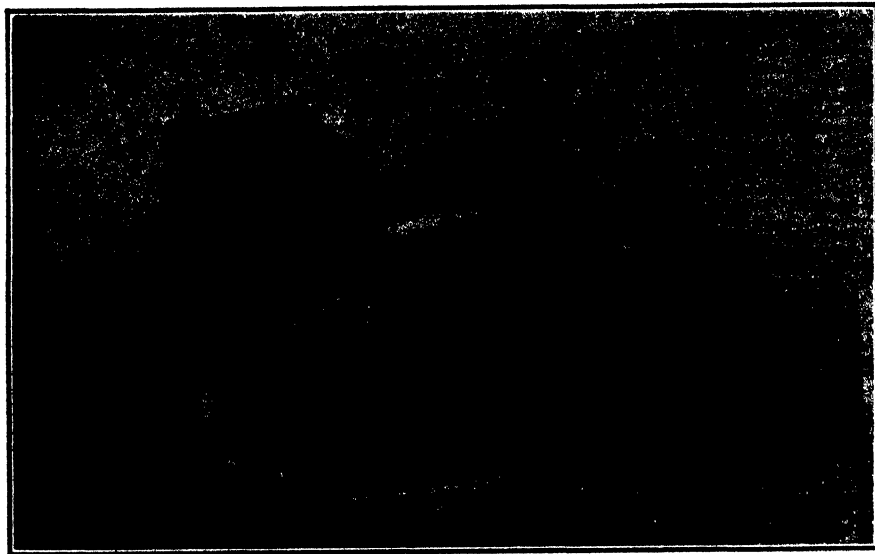
Some of the differences between normal blood and blood containing carbon monoxide are readily evident, such as the colour (which is invariably but rather unhappily described as cherry-red), whilst others are revealed only by the spectroscope. The colour of the blood, at least in the cadaver, is not specific as is so generally supposed, and it is essential in the absence of other positive evidence that spectroscopic examination should be made before arriving at a conclusion.

Normal blood shows two absorption bands between the D and E lines, and blood containing carbon monoxide will appear to exhibit identical features unless an instrument of precision is employed, when, especially if the percentage saturation of the blood is high and the spectrum of a normal blood is viewed simultaneously, careful observation will reveal that the bands of carboxyhaemoglobin are very slightly nearer the violet end of the spectrum. The spectroscopic difference is too slight to be diagnostic, unless the Hartridge Reversion Spectroscope is employed.

A chemical method of differentiation is available by observing the effect of the addition of a reducing agent, such as ammonium sulphide, when, in theory, with a normal blood the two bands of oxyhaemoglobin merge into the one broad band of haemoglobin, and in blood containing carbon monoxide the bands of carboxyhaemoglobin remain unchanged. This chemical method of differentiating the bands has been transcribed from one text-book to another, and the accounts nearly all give the same impression of a process without difficulties or limitations. In practice, in my hands, this test has frequently yielded results which were open to doubt, except with blood almost saturated with carbon monoxide, even when carried out with simultaneous treatment and observation of a normal blood control. This is only to be expected, for in the presence of a reducing agent the stable absorption bands of carboxyhaemoglobin are masked to a less or greater extent by the broad band of haemoglobin from reduction of oxyhaemoglobin according to the percentage saturation of the blood.

With the Hartridge Reversion Spectroscope it is possible in a few minutes not only to detect carbon monoxide in blood with certainty, even in small amount, but also to determine the percentage saturation, and, therefore, to express an opinion

whether this was the cause of illness or death. In life the gas is rapidly eliminated as soon as the person is removed from the poisonous atmosphere, but specimens of blood taken from an individual will retain the carbon monoxide for weeks if these are stored under suitable conditions.



THE HARTRIDGE REVERSION SPECTROSCOPE

Fig. 1.

**THE HARTRIDGE REVERSION SPECTROSCOPE.**—The spectroscope is shown in Fig. 1; it is quite a small instrument, measuring at the longest diagonal of the base only about 14 cm. A source of light must be provided at the collimating lens (seen projecting behind the micrometer screw). The lighting unit (which may be obtained with the instrument) is conveniently a 60 c.p. electric lamp housed in one box arrangement superimposed on another which contains an adjustable mirror and has one side (facing the spectroscope) made of ground glass. I had this reconstructed, and the complete apparatus, with the spectroscope adjustable in position, mounted on a miniature table, 35 cm. high. Interposed between the source of light and the spectroscope is a holder for the cell containing the blood under examination.

The cells used are 40 mm. square at the face and are of the type made by cutting a U-shaped piece from a solid piece of glass. Those made for me are 5 mm. thick; the depression is a true semi-circle (17 mm. in diameter, at the bottom) and has an extreme height of 30 mm. The diameter is just sufficient to cover completely the collimating lens and enables an examination to be made, if necessary, with the absolute minimum quantity of sample—a single drop of blood.

The principle of the instrument is the utilisation of the fact already referred to, that in blood containing carbon monoxide the absorption bands are situated

slightly nearer the violet end of the spectrum and, in addition, that the extent of this difference in wave-length is related to the percentage saturation. To enable this difference to be measured more accurately the effect is doubled by the simple and ingenious expedient of employing two spectra in reverse directions. With the instrument at zero the field of the spectroscope in observation of a normal blood is shown (Fig. 2) in *a*, and with blood containing carbon monoxide in *b*. By turning the micrometer screw the bands, in either case, may be brought into the basal position shown in *c*, and a record obtained, directly in Ångström Units, of the difference in wave-length between a normal blood and a blood sample containing carbon monoxide. This difference Hartridge terms the "span."

**THE DETERMINATION OF THE SPAN OF A BLOOD SAMPLE.**—The instrument should be used in a dark-room. The mirror of the lighting unit and the spectroscope having been adjusted to give the essential uniform illumination of the spectra, the reading for a normal blood is first obtained.

The normal blood should be human and from an individual who is not a heavy smoker; Hartridge<sup>6</sup> has found that blood from such subjects may be saturated with carbon monoxide to the extent of 6 per cent. Animal blood is to be avoided, as the intestinal gases of herbivora are stated to contain carbon monoxide; Hartridge<sup>7</sup> has found carbon monoxide in sheep's blood. The blood is diluted with distilled water\* to such a degree that trial observations show the bands to be visible distinctly and the distance between them to be about the same as the width of the alpha band. For this purpose the small quantity of diluted blood required is pipetted (using a drawn-out glass tube with rubber teat attached) into or from a cell which is, of course, interposed between the instrument and the source of light. It will be necessary, probably, to focus; this is done by adjustment of the milled screw on the collimating lens. Once the position of this has been found it must not be moved again before examination of the suspected sample. Observation is made at the eyepiece seen on the extreme left of Fig. 1; it is fitted with a shield, not shown. While these trial observations are being made, the micrometer screw (seen on the extreme right of the illustration) should be at zero. The knife edge in the centre foreground is a coarse adjustment, but this is not used; it is placed exactly at 3 and is thereafter ignored, except to see that it remains in that position.

These preliminaries having been completed, the reading for normal blood is now determined by turning the micrometer screw until the two alpha bands are in line (Fig. 2, *C*), and then, when this position has been attained, noting the figure on the screw. It is necessary to take the average of ten readings; a certain degree of variation will be found between the separate figures, but the average of a series should be within one or two digits of another series of observations on the same blood. The most concordant results are obtained by making the adjustment quickly and without hesitation. The screw is graduated into 100 divisions, each

\* The effect of adding distilled water is to burst the envelopes of the red blood corpuscles (hence the turbidity produced) and to cause the contained haemoglobin to pass into solution; this phenomenon is known as "laking."



**PREPARATION OF CALIBRATION CURVE.**—For the preparation of the curve it is necessary to establish four points: the span of blood saturated with carbon monoxide to the extent of (i) 25, (ii) 50, (iii) 75, and (iv) 100 per cent. with carbon monoxide. Stock quantities of diluted normal blood (N) and blood saturated with carbon monoxide (S) must first be prepared. For N, a small quantity of blood is placed in a stoppered bottle, and distilled water is added to this until observation of a portion shows the degree of dilution to be such as has been specified in the foregoing. For S, coal gas is passed through a similar quantity of blood in another stoppered bottle, which is shaken from time to time; this blood is then diluted as requisite and afterwards again treated with coal gas to make certain of complete saturation.

Two 5 mm. cells are employed, face to face. The reading for normal blood, which has to be deducted from each reading of blood containing carbon monoxide, is obtained by filling one cell with dilution N and the other with distilled water and taking the average of a series as already described.

(i) *Span for 25 Per Cent. Saturation.*—In a test tube are placed 7.5 c.c. of dilution N and 2.5 c.c. of distilled water; in another 2.5 c.c. of dilution S and 7.5 c.c. of distilled water. Both dilutions are mixed in their separate tubes, and a quantity from one is pipetted into one cell, and from the other into another cell. With the two cells face to face as before, the average of a series of readings is obtained, and this, less that for the normal blood, is the figure required.

The other figures are determined by the same procedure, except that the preparation of the test tube dilutions is varied as noted below.

(ii) *Span for 50 Per Cent. Saturation.*—Five c.c. of dilution N and 5 c.c. of distilled water in one; 5 c.c. of dilution S and 5 c.c. of distilled water in the other.

(iii) *Span for 75 Per Cent. Saturation.*—The dilution N (2.5 c.c.) and 7.5 c.c. of distilled water in one; 7.5 c.c. of dilution S and 2.5 c.c. distilled water in the other.

(iv) *Span for 100 Per Cent. Saturation.*—Distilled water alone in one; dilution S alone in the other.

On plotting out the span figures a smooth curve should be obtained, and this is extended to the zero point. The calibration curve once constructed is available permanently for immediate conversion of the span of a blood sample into terms of percentage saturation.

**NOTE ON THE PRESENCE OF NITRIC OXIDE HAEMOGLOBIN.**—The possibility of NO-haemoglobin, formed after death, being mistaken for CO-haemoglobin in the examination of blood has been discussed by Banham, Haldane, and Savage,<sup>8</sup> following a case where the individual had not been exposed to carbon monoxide, yet the blood of whom, post-mortem, responded to certain of the usual tests for CO-haemoglobin; the spectroscopic examination, it is important to note, was not carried out, though they considered that had this been done it would have confirmed the apparent presence of carbon monoxide. The opinion was expressed

that the actual cause of death was broncho-pneumonia due to an infecting organism which produced nitrite.

These authors state that the double-banded spectrum of NO-haemoglobin is similar to that of CO-haemoglobin and oxyhaemoglobin, except that the bands are much less sharply defined than those of oxyhaemoglobin, and somewhat less sharply than those of CO-haemoglobin; the NO-haemoglobin band in the yellow extends also to a slight distance on the red side of the D line. The positions of the alpha and beta bands of the three haemoglobin compounds under discussion have been determined by Hartridge,<sup>9</sup> and the wave-lengths of these are given below.

			Alpha band.	Beta band.
			A.U.	A.U.
Oxyhaemoglobin	..	..	5768	5398
CO-haemoglobin	..	..	5714	5360
NO-haemoglobin	..	..	5785	5418

Examination of these figures shows that there is no reason why NO-haemoglobin should be mistaken for CO-haemoglobin if the reversion spectroscope is employed, for if NO-haemoglobin is present, the span will be a minus instead of a plus figure.

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NOTE.—A valuable summary of the literature on carbon monoxide poisoning (with a bibliography of 195 references) is given by R. R. Sayers and Sara J. Davenport in *Review of Carbon Monoxide Poisoning*, Public Health Bulletin, No. 195, Washington, D.C., United States, Government Printing Office, 1930.

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#### DISCUSSION.

Dr. ROCHE LYNCH said that he was very glad that Mr. Frederick had brought this instrument to the notice of the Society, as he thought it was not at all well known. Most analysts had to examine blood at times, and there was no doubt that this instrument provided the most suitable method of analysis. In his experience it was capable of much greater accuracy than the Haldane method. With the Hartridge instrument one could work with low percentages and deal with even 4 or 5 per cent. of carbon monoxide; this would be useful in cases of chronic

carbon monoxide poisoning. There was a great deal of this about, although it was not generally recognised. In many households there were gas jets, gas rings, geysers, etc., constantly alight and having no flues, and these were all giving off carbon monoxide into rooms where people spent a great deal of time. He believed a considerable number of minor ailments were due to people inhaling carbon monoxide in this way. Mr. Frederick had pointed out that 80 per cent. saturation would cause death for certain, and he (Dr. Roche Lynch) was inclined to think that probably 60 to 65 per cent. could cause death. However, it must be remembered that if the patient were taken out in a moribund condition and lived for a few hours it was surprising how quickly the percentage in the blood fell, and very possibly only about 15 per cent. would be found. Also, there were cases of carbon monoxide poisoning where the patient lived for two or three days and then died. Here two factors had to be considered—the factor of the percentage in the blood and the factor of the damage done to the tissues as a result of exposure to the deoxygenated blood. Regarding the figures for suicide from coal gas, he remarked that this was a very easy way of terminating life and, although one would like to see an even greater tightening of the Poisons Act, the narcotic poisons available to the public were really very few, and consequently coal gas helped to bring up the suicide figures considerably.

Sir BERNARD SPILSBURY thanked Mr. Frederick for his observations on nitric oxide haemoglobin, which tended to confirm his own opinion. He must plead guilty to having used the Haldane method. Dr. Haldane claimed to read to within 3 per cent., and he (Sir Bernard) thought that he could read to within 5 per cent., which, of course, was satisfactory where large amounts were concerned. He admitted that the test certainly required delicacy of judgment in matching the sample under examination with the standard. His investigations had always been made upon the bodies of persons found dead. In these cases the blood was difficult to examine owing to its coagulation after death, and he wondered if the same difficulty were likely to arise with the Hartridge reversion spectroscope. The figures given by Henderson for a fatal result seemed to be unreasonably high. Of course, here again one had to take into consideration the difference in the results obtained by the two methods, but he, himself, generally found the fatal figure to be about 60 per cent.; it had reached 70, but never approached 80 per cent. The figures given were, presumably, for normal people, whereas people not in normal health succumbed more rapidly. He agreed that the Hartridge reversion spectroscope was a great improvement for lower saturations of the blood and was very useful in cases of chronic carbon monoxide poisoning. Quite recently a medical friend had asked him to investigate certain illness, the cause of which he could not discover. Children were always ill when living in the house, although quite well when away. On examining the premises, Sir Bernard had found in a basement kitchen a destructor used for getting rid of household refuse. It was a coke furnace, standing near the kitchen stove, with a flue from the top of the furnace to the top of the chimney. However, there was a loose-fitting lid covering the hole into which the rubbish was put, and gases could very easily escape from this into the room. A mouse was placed on the kitchen mantelpiece, and in three days it died, death being proved to be due to carbon monoxide poisoning. Such instances must be fairly common in ordinary households, and, in these days of almost universal motoring, also in many badly ventilated garages. For these cases the Hartridge method of investigation would be of very great advantage.

Mr. W. J. A. BUTTERFIELD said that it was very interesting to hear an account of this instrument, although he had no experience of its use. The figures for the



number of deaths from coal gas poisoning during 1918 and 1928, were probably fairly comparable as regards suicides, but he was quite sure, from a study of the statistics which he made some years ago for the purpose of a report to the Board of Trade published in 1924, that it was very misleading to take a single year's accidents and compare them with any other single year ten years later. They varied very much from year to year, and to get a fair comparison he was sure that three consecutive years, at least, should be taken. For instance, comparison of 1917, 1918 and 1919 with 1927, 1928 and 1929 would be much fairer. One got an enormous difference by picking out individual years ten years apart; if one took the next year in each case one got entirely different figures. It had been suggested that the increased number of accidents was due to the increase of the proportion of carbon monoxide in the gas. As regards 1918 and 1928 the carbon monoxide in gas in this country was much higher in 1918 than in 1928, owing to the emergency measures adopted during the war years to produce gas to keep up the supply. During some of these years the carbon monoxide was much higher than it had ever been since. How misleading comparisons of single years could be might be appreciated from the facts that the mean proportion of carbon monoxide in all the gas supplied in Great Britain was 15.2 per cent. in 1921, when the number of accidental deaths was 126, and 14.4 per cent. in 1922, when the accidental deaths rose to 203. Regarding the susceptibility to small proportions of carbon monoxide in the air breathed, any reference to headaches resulting therefrom must be referred to the previous experience of the individual; otherwise, one might be misled very much. People living in an atmosphere free from carbon monoxide were more susceptible to small doses. He gave one illustration—the drivers and stokers of the engines on the old Metropolitan Railway in the time of the steam trains were in the tunnels a considerable time, inhaling carbon monoxide which (between King's Cross and Edgware Road) incapacitated anyone who tried to walk through the tunnel, quite apart from the sulphur dioxide. These men felt quite well, and examination of their sick-club books showed them to be a very healthy set. The same thing was experienced in gas works and in garages. Mr. Butterfield added that he had mentioned this paper to Dr. Haldane, but as the title suggested that it was simply a description of the instrument, Dr. Haldane had thought it unnecessary to postpone a visit to the North in order to be present at the meeting. With regard to the treatment with carbon dioxide mixed with oxygen, which was claimed to be of American introduction, he did not think Prof. Henderson claimed it as originating in America. The actual origin was due to observations in war time in Europe, and the theory was certainly known in this country before the practice was developed and brought into general use in America.

Mr. W. PARTRIDGE said that treatment with oxygen mixed with carbon dioxide was claimed to hasten respiration. Speaking from memory, he was under the impression that about ten years ago an American committee, dealing with the proportion of carbon monoxide in the streets, had found that the addition of carbon dioxide reduced the toxicity of carbon monoxide. The years 1918 and 1928 were bad ones to be taken for accidental deaths, because the peak year was about 1922 or 1923, and at the later date he believed the companies were using drawn pipes and not seamed tubing for gas. Dr. Roche Lynch had mentioned that fewer poisons were available in 1928 than in 1918. He (Mr. Partridge) did not think that many of the additional poisons scheduled as dangerous in the ten years mentioned were generally available to the public.

Mr. FREDERICK, replying, said that there was little he need say in reply, since the remarks made were not so much in the nature of criticism as valuable

contributions to our knowledge of the subject of the paper. In any discussion of poisoning by carbon monoxide there was a tendency for undue importance to be attached to the question of suicide by this means; suicide statistics were influenced by so many factors that he preferred not to pursue this aspect of the subject. He was sorry that Dr. Haldane had not been present, as his authoritative remarks would have added still further to the interest of the discussion. With regard to the treatment of carbon monoxide poisoning by a mixture of oxygen and carbon dioxide, this was really outside the scope of the paper, and had only been mentioned as a matter of general interest.

Communications on the foregoing paper:—

### THE INVESTIGATION OF CARBON MONOXIDE POISONING.

By J. S. HALDANE, C.H., F.R.S.

As Mr. Frederick's paper conveys the impression that in the examination of blood from suspected cases of carbon monoxide poisoning the use of spectroscopic methods, and particularly of the Hartridge reversion spectroscope, is essential, I should like to point out that this is not the case. The colorimetric method, as applied to diluted blood, requires no special apparatus, can be made with a single drop of the blood, and, when it is made quantitative, gives very exact information as to the percentage saturation of the haemoglobin with carbon monoxide. I do not think that an analyst requires to use a more complicated method; it is this simple method which I have used exclusively in the numerous investigations which I have made of carbon monoxide poisoning or in which carbon monoxide was used as a physiological reagent. In cases of suspected nitrite poisoning the colorimetric method gives information at once if the blood sample is taken during life, when much methaemoglobin is present; and after death the presence of nitric oxide haemoglobin is at once revealed by the colour of the clot formed on boiling.

### THE PREPARATION OF NITRIC OXIDE HAEMOGLOBIN.

By H. HARTRIDGE, M.D., F.R.S.

Nitric oxide haemoglobin was prepared by Gamgee by passing nitric oxide gas through blood or a solution of haemoglobin. Since nitric oxide reacts with oxygen to form nitrogen peroxide, and since this reacts with water to form nitric acid which would change the haemoglobin to acid haematin, oxygen must be excluded during the reaction of the nitric oxide with the blood or haemoglobin, or alkali must be added previous to the reaction so as to neutralise the nitric acid which will be formed. In order to exclude oxygen a stream of neutral gas, such as nitrogen or hydrogen, may be passed through the apparatus in which the nitric oxide is to be generated and through the blood which is to react with it. If this neutral gas is oxygen-free, the oxygen in combination with the haemoglobin may be dissociated from it by warming and shaking the haemoglobin during the passage of the neutral gas. Hufner prepared nitric oxide haemoglobin by first passing pure carbon monoxide gas through the apparatus and the blood. This gas displaced the oxygen both from the apparatus and the blood, with the formation of carbon monoxide haemoglobin. This compound, on passing the nitric oxide gas, was then decomposed into nitric oxide haemoglobin, with the liberation of the carbon monoxide. Nitric oxide haemoglobin can be more readily obtained by adding

to blood a solution of a nitrite of an alkali metal and a suitable reducing agent. Dilute solutions of sodium nitrite and ammonium sulphide are commonly used.

**THE PROPERTIES OF NITRIC OXIDE HAEMOGLOBIN.**—It is a red crystalline compound (Hermann). Its solutions are less orange than those of oxyhaemoglobin, but are more orange than those of carbon monoxide haemoglobin. Two absorption bands are present in the visible spectrum, which superficially resemble the oxyhaemoglobin bands in position but are more diffuse. A comparison of the position of its absorption bands with those of oxyhaemoglobin and carbon monoxide haemoglobin, with the reversion spectroscopy, shows that they are on the long wave-length side of those of oxyhaemoglobin; that is, on the opposite side to those of carbon monoxide haemoglobin (see Table at the end of Mr. Frederick's paper). Experiments on the stability of nitric oxide haemoglobin show that, whereas nitric oxide can displace carbon monoxide from its combination with haemoglobin, the compound thus produced is not so stable, but tends to change spontaneously into methaemoglobin. On heating a solution of carbon monoxide haemoglobin, coagulation commences at about 65° C., whereas on heating a solution of nitric oxide haemoglobin to 50° C., it spontaneously changes to alkaline methaemoglobin, which then coagulates.

## The "Rope" Spore Content of Flour and its Significance.

By A. J. AMOS, B.Sc., A.I.C., AND D. W. KENT-JONES, PH.D., B.Sc., F.I.C.

(Read at the Meeting, May 6th, 1931.)

**INTRODUCTION.**—There are only two diseases, other than mould infections, to which wheaten bread is known to be liable, and, fortunately, neither of them is of frequent occurrence. The disease giving rise to the so-called "bleeding bread" occurs so rarely that it has never occasioned an exhaustive investigation, whereas the "rope" disease, although by no means common, has been extensively studied by a large number of workers.

It has been definitely established that the bacteria responsible for the production of "ropiness" in bread are members of the *mesentericus* group. *B. mesentericus vulgatus* (Flügge) has been isolated from "ropy" bread by Kratschmer and Niemilowicz (1889), Uffelmann (1890), Watkins (1906), and Kent-Jones and Amos (1930); *B. mesentericus fuscus* by Watkins (1906) and Kent-Jones and Amos (1930); *B. mesentericus niger* by Biel (1896) and Lloyd, Clark and McCrea (1921); *B. mesentericus ruber* by Kent-Jones and Amos (1930). Other organisms that have been isolated are *B. panificans* by Laurent (1884), *B. liodermos* by Uffelmann (1890) and *B. mesentericus panis viscosus* I and II by Vogel (1897), although it is probable that some of these organisms are identical with some of those given above. Lloyd, Clark and McCrea (1921) isolated five different types, which they labelled A to E, but from the table of cultural characteristics which they give it seems extremely probable that their type B was *B. mesentericus vulgatus*, whilst

their type A was possibly identical with the *B. mesentericus ruber* reported by Kent-Jones and Amos (1930).

It is remarkable, however, in view of the large amount of published literature on the subject of "ropy" bread, that so little attention has been paid to the extent to which flours are contaminated with these causative bacteria.

It seemed to us that a knowledge of such contamination might not only throw more light on the development of the disease, but might also be of importance in outbreaks of "rope" in deciding to what extent the blame rested with the miller or the baker.

Lloyd, Clark and McCrea, in the paper cited above, describe a method for the enumeration of heat-resistant spores in flour and give a number of results obtained by applying the method to various samples of wheat and flour. The American Association of Cereal Chemistry (1928), in their book of standard methods, describe a method for the determination of the bacterial spores in flour which is essentially the same as the above. In our opinion, however, these methods are open to serious criticism, and we were unable to obtain consistent results with them.

The only other method known to us for determining quantitatively the contamination of flours with rope organisms was one personally communicated to us by Dr. Hoffman, of New York, and briefly reported upon in our previous paper (Kent-Jones and Amos, *ANALYST*, 1930, 55, 248). We have now examined this method more critically, and, in our view, it does not yield sufficiently satisfactory results. However, we preferred it to those mentioned above, and hence have used it as the basis for our technique.

EXPERIMENTAL.—All the three methods referred to are dependent upon the fact that the members of the *mesentericus* group are characterised by the formation of spores which are extremely resistant to heat. Lloyd, Clark and McCrea shake 100 grms. of flour with 300 c.c. of sterile water in a plugged flask and then add 1 c.c. of the resulting suspension to each of four tubes of melted nutrient agar. The tubes are heated in boiling water for 20 minutes and the contents are then poured into sterile Petri dishes. Our own experiments lead us to conclude that this method suffers from the following serious faults:

- (1) The method of shaking (*viz.* in a plugged flask, where violent shaking is impossible), especially with a water to flour ratio as small as 3 to 1 does not lend itself to the complete detachment and uniform distribution of the bacteria.
- (2) The heating at 100° C. often results in the formation of agglomerates due to the gelatinisation of the starch, because the viscosity of the melted agar does not allow thorough mixing of this medium and the added suspension. These agglomerates tend to entrap organisms and thus cause low counts.
- (3) In pouring the plates, even if this is performed at 100° C., a certain amount of both the flour and the agar is left in the tube.

These remarks apply also to the method of the American Association of Cereal Chemists which only differs in that 500 c.c. of water are used instead of 300 c.c.

In Hoffman's method the primary suspension is prepared by shaking 2 grms. of the flour with 94.6 c.c. of sterile water and 5 grms. of sterile sea sand. Volumes of this suspension varying from 4.8 c.c. to 0.192 c.c. and corresponding with weights of flour varying from 1/10 to 1/250 gm. are added to 10 c.c. tubes of standard nutrient broth and the tubes then heated for 30 minutes in an Arnold steriliser. The tubes are incubated at 37.5° C. for 48 hours, and at the end of this time are reported as positive or negative according to whether they show a pellicle or not. The number of rope spores per gm. of flour is taken as being equal to the reciprocal of the highest dilution giving a positive result.

As previously mentioned, we were unable to obtain sufficiently consistent results by the use of this method; in some cases, where duplicate suspensions were prepared, the set of tubes from one suspension were all negative, whilst the other set indicated the presence of as many as 40 spores per gm.

As the quantity of flour used (2 grms.) was very small, the effect of increasing this to 10 grms. was investigated. There was some improvement in the results, but the agreement was still not sufficiently good.

During incubation the added flour lies at the bottom of the tubes, of course, and it seemed possible that this sediment, in some cases, might be sufficient to entrap organisms and prevent them from forming a pellicle. Some tubes which were negative at the end of 48 hours were accordingly well shaken and replaced in the incubator; at the end of 16 hours a number of them were positive. This result indicated the further necessity of thoroughly shaking each tube *immediately* before it was placed in the steriliser, as otherwise, especially with the tubes containing 1/10 and 1/20 gm. of flour, the gelatinisation of the starch resulted in the formation of a tenacious mass which could not be disintegrated and distributed throughout the liquid by subsequent shaking. On thoroughly shaking the tubes immediately before placing them in the steriliser, the gelatinised particles were of very small size, and subsequent shaking produced a uniform mixture.

This shaking of the tubes introduced another source of error. Some tubes contained 14 c.c. of liquid (10 c.c. of broth and 4 c.c. of added suspension), and it was found to be almost impossible to mix thoroughly the contents of such tubes without wetting the plugs. In several cases where this had occurred the tube gave a negative result, but on the under side of the wetted plug was a slight growth of rope organisms. It was thought to obviate this source of error by the employment of wider tubes (1 inch diameter instead of 5/8 inch), but this modification caused the results to be less consistent. The explanation probably is that, in the narrower tubes, the film receives artificial support by adherence to the walls of the tube at an earlier stage of its formation; in the wider tubes the frail pellicle, before reaching the walls, may become dislodged and sink to the bottom.

The necessity for retaining the narrower tubes meant that a smaller quantity of broth would have to be used, but, in order to prevent undue dilution through the addition of comparatively large amounts of suspension, it became necessary to increase its strength.

The only other modifications introduced consisted in increasing the quantity of both the flour and the sand to 20 grms. and shaking them in 400 c.c. of sterile 0.5 per cent. sodium chloride solution instead of in 100 c.c. of water.

One point of great importance that emerged from these investigations was that, for the results to be reliable, the positive tubes when finished with must be cleaned by a special method (such as that given later) before being used again. We found that heating the positive tubes in a steam steriliser for 30 minutes, cleaning out the contents with hot water and a brush, plugging and heating at 150° C. for one hour and a half, and then filling with fresh broth and sterilising for 10 minutes on three successive days did not, in some cases, result in the death of all the contaminating rope spores; after several days' storage a number of the tubes so treated showed a pellicle.

**METHOD.**—The details of the method finally adopted are as follows:—The bottles, pipettes and measuring cylinder are sterilised by heating at 150° C. for two hours. The 0.5 per cent. sodium chloride solution is sterilised by boiling in a plugged flask for one hour. Four hundred c.c. of the sodium chloride solution are measured into a sterile 16-oz. glass-stoppered bottle and 100 c.c. into a sterile 8-oz. glass-stoppered bottle. Twenty grms. of purified silver sand are heated to redness in a platinum capsule for some minutes, and then poured, while hot, into the liquid in the 16-oz. bottle. Twenty grms. of the flour are weighed out on a sterile watch-glass and transferred by means of sterile paper to the 16-oz. bottle. This bottle is then shaken vigorously for two minutes. Volumes of 2 c.c. and 1 c.c., respectively, of the suspension are then added immediately, by means of a sterile 1 c.c. pipette, to 5 c.c. tubes of the broth (for preparation of the broth, see later). The bottle is re-shaken once or twice and, by means of a sterile pipette, 20 c.c. of the suspension are added at once to the liquid in the 8-oz. bottle, and this bottle then well shaken. By means of a fresh sterile 1 c.c. pipette, volumes of 4 c.c., 3 c.c., 2.5 c.c., 2 c.c., 1.7 c.c., 1.5 c.c., 1.3 c.c., 1.2 c.c. and 1 c.c. respectively of this dilution are then added to 5 c.c. tubes of the broth. The bottle is shaken once or twice before the withdrawal of each of the above quantities. Each tube is taken individually, the contents thoroughly mixed, and the tube *at once* placed in a bath of boiling water, so that the level of the water is above the level of the liquid in the tube. The tubes are allowed to remain in the bath for 20 minutes, and during this time the water is kept boiling. On removal of the tubes from the bath their contents are thoroughly mixed and they are then placed in an incubator kept at 37° C. The contents of the tubes are well mixed three or four times during the first 24 hours' incubation. At the end of 48 hours' incubation the tube is reported as positive or negative according to whether the contained broth shows a pellicle or not. The number of rope spores per gm. is taken as the reciprocal of the

smallest fraction of a grm. of flour giving a positive result. If, as sometimes happens, a negative result is obtained with a quantity of flour greater than the smallest amount giving a positive result, then the number of spores per grm. is taken as the reciprocal of the quantity next greater than the smallest amount giving a positive result. The quantity of flour corresponding with the various volumes used is as under:

2	c.c. of primary suspension	≡	1/10	grm. flour
1	c.c. "	≡	1/20	" "
4	c.c. of 20 : 100 dilution	≡	1/30	" "
3	c.c. " " " "	≡	1/40	" "
2.5	c.c. " " " "	≡	1/48	" "
2	c.c. " " " "	≡	1/60	" "
1.7	c.c. " " " "	≡	1/70	" "
1.5	c.c. " " " "	≡	1/80	" "
1.3	c.c. " " " "	≡	1/92	" "
1.2	c.c. " " " "	≡	1/100	" "
1	c.c. " " " "	≡	1/120	" "

If the flour contains more than 120 spores per grm. a further dilution is made.

*Cleaning of Used Tubes.*—The tubes are heated in a steam steriliser for at least 30 minutes. The plugs are then removed, the contents discarded, and the tubes well washed out with hot water, a test-tube brush being employed. The tubes are then placed in a bath of mercuric chloride solution (1 : 1000) and allowed to remain there for some hours. On removal from this bath, the tubes are washed out ten times with water, drained, plugged and heated at 150° C. for one hour and a half.\*

*Preparation of Nutrient Broth.*—Ten grms. of B.D.H. bacteriological peptone, 5.4 grms. of "Difco" beef extract and 9 grms. of A.R. sodium chloride are dissolved in a litre of distilled water, and the pH adjusted to 7.2 to 7.3. The solution is boiled and filtered, and the filtrate made up to one litre. The medium is distributed in 5 c.c. quantities in sterile test-tubes of 5/8 inch diameter and sterilised for ten minutes on three successive days in a steam steriliser. In order to make certain of the complete absence of rope spores in the sterilised tubes of broth, they are placed in a blood-heat incubator for 48 hours; any of the tubes that show pellicle formation are discarded.

It is not to be expected, of course, that attempts to enumerate living organisms in a substance will furnish results as consistent as those obtained in the

\* Since the reading of this paper it has been suggested to us that the use of mercuric chloride as a disinfectant may result in the inhibition of growth when the tubes are again employed, owing to the imperfect removal of this substance.

Whenever a negative result was obtained in the above experiments, however, we inoculated the broth with a pure culture of *B. mesentericus vulgatus*, and in every case a pellicle was subsequently formed. This showed that the non-formation of a pellicle in the test was not due to the bactericidal action of traces of mercuric chloride.

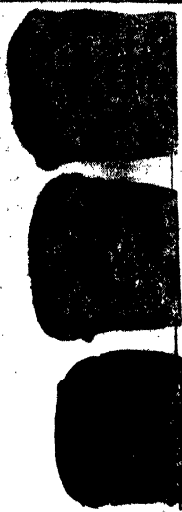
The cleaned tubes can also be tested by filling them with litmus-lactose-peptone water, inoculating the medium with *B. coli*, and then incubating the tubes at 37° C. for 48 hours. The production of acid indicates that the disinfectant has been successfully removed.



A      B  
FIG. 1.



A      B      C  
FIG. 2.



A      B      C  
FIG. 3.



A      B      C  
FIG. 4.



A      B      C  
FIG. 5.

LOAVES INFECTED WITH "ROPINESS."





determination of definite chemical compounds. When, as in the case under consideration, the organisms to be enumerated are normally present in small numbers, and the substance itself is particularly difficult to deal with, then the variation in results to be expected is still greater. For these reasons we consider it advisable always to prepare duplicate sets of tubes from the suspension. Often, of course, the two sets do not yield the same figure, but we consider that the results obtained under these conditions are, in view of the nature of the problem, satisfactorily consistent. As examples, we give the following typical results:

TABLE I.

		Quantity of sample in Grms.											Spores per gram.
Reference.	Tubes.	1/10	1/20	1/30	1/40	1/48	1/60	1/70	1/80	1/92	1/100	1/120	
A	1st series	+	+	+	+		—	0	0	0	0	0	} 40
	2nd „	+	+	+	+		—	0	0	0	0	0	
A (same sample week later)	1st series	+	+	+	—	—	0	0	0	0	0	0	} 30
	2nd „	+	+	—	+	—	0	0	0	0	0	0	
B	1st series	0	0	0	0	+	+	—	—	0	0	0	} 60
	2nd „	0	0	0	0	+	+	—	—	0	0	0	
C	1st series	—	—	—	—	0	0	0	0	0	0	0	} <10
	2nd „	—	—	—	—	0	0	0	0	0	0	0	
D	1st series	+	+	+	+	0	+	+	+	—	—	0	} 70-80
	2nd „	+	+	+	+	0	—	+	+	—	—	0	
E	1st series	0	0	0	+	+	+	0	—	0	+	+	} at least 100
	2nd „	0	0	0	+	—	+	0	+	0	+	+	
F	1st series	+	—	+	—	—	0	0	0	0	0	0	} 20
	2nd „	+	+	—	—	—	0	0	0	0	0	0	
G	1st series	0	0	0	+	—	+	+	+	—	—	0	} 60-70
	2nd „	0	0	0	+	+	—	+	—	—	—	0	
H	1st series	0	0	+	+	+	—	+	+	—	0	0	} about 60
	2nd „	0	0	+	+	—	+	—	—	—	0	0	
J	1st series	+	+	—	+	—	+	—	—	0	0	0	} 40-50
	2nd „	+	+	+	—	+	—	—	—	0	0	0	

0 = tube omitted from series.

*B. subtilis* is another organism which sometimes occurs in flour and, if present, will form a pellicle on the broth. Since experiments we have performed have shown that this organism does not turn bread ropy, it may be advisable, when the spore count is high, to determine whether the pellicles are due to this organism and not to those of the *mesentericus* group. A quick way of doing this is to prepare agar slant cultures from the pellicles; the form of the resulting growth will indicate which bacteria are present. *B. mesentericus vulgatus* forms a greyish-white growth which is at first moist and "blister"-like in appearance, but later becomes drier and finely wrinkled. *B. subtilis* gives a whitish, spreading growth,

the edges of which, viewed under a low power, appear as an interwoven mass of fine hairs.

**THE SIGNIFICANCE OF THE "ROPE" SPORE CONTENT OF FLOUR.**—Having established a satisfactory method of determining the rope spore content of flours, we decided to investigate the connection existing between this factor and the development of the "rope" disease in bread.

Watkins (1906) considered that the number of rope organisms in the flour was of primary importance, and Hoffman, in the private communication cited above, also concludes that this is the most important single factor. He states, further, that 40 spores per grm. is a reasonable limit, but that counts beyond this figure are unsafe.

In our preliminary experiments we made loaves from flours of different spore contents and stored these loaves in a warm, moist atmosphere. We confirmed that, generally speaking, the greater the spore content of the flour, the more rapidly did rope develop in the loaf, provided, as will be emphasised later, that all the other conditions were equal.

We then made an appeal, through trade journals, for any baker who had an outbreak of rope to send us samples of the bread and of the flour from which the bread was made. These samples, and others, which were causing no complaints, we examined by our method, and the results obtained are given in Fig. 2.

TABLE II.

Reference.	Type of flour.	Complaint.	Number of spores per grm.
PC1	Wheatmeal	Bread badly ropy	60
PC2	"	None	<40
WT	"	Bread badly ropy	30
WTR	Bakers' grade	" " "	30
WTF	" "	" " "	<10
WTM	" "	" " "	<10
F1	" "	" " "	30
F2	" "	" " "	<20
B1	" "	" " "	30
B2	" "	" " "	30
CS1	" "	None	120
CS2	" "	"	160
A	" "	"	80

These results are surprising in that they appear to be contrary to the indications given by our preliminary experiments with regard to the influence of the spore content of the flour upon the incidence of the disease in the final bread. With one exception, all the bread in which the disease had developed had been made from flours which did not contain more than 30 spores per grm.; in two cases the number of spores per grm. was less than 10. On the other hand, two flours which contained over 100 spores per grm. had caused no complaints, although they had been sent out to a number of commercial bakeries during the summer months.

These facts make it evident that, in the case of commercial bread, the number of rope spores in the original flour is not the most important factor in the development of the disease. The explanation of the apparent discrepancy is as follows:

In our own baking tests, all the flours had been subjected to exactly the same procedure during fermentation and baking (see *Modern Cereal Chemistry*, 2nd Edn., p. 174), and the final loaves had all been stored under identical conditions. Thus, of all the factors liable to affect the incidence of the disease, the only one that varied was the spore content of the flour. In commercial practice, however, the systems of fermentation, times and temperatures of baking and conditions of bread cooling and storage which prevail are as varied as the spore contents of the flours employed.

We concluded, therefore, that whether bread becomes ropy or not under commercial conditions does not depend so much upon the number of rope spores in the original flour, but is governed almost entirely by those factors which accelerate or retard the development of the organisms in the final bread. That is, a flour may have a low spore content and yet yield ropy bread because either the bread itself or the conditions of cooling and storage, or both, are particularly suitable for the development of the disease. On the other hand, a flour may contain a large number of spores and yet furnish satisfactory bread because these spores are unable to develop sufficiently on account of the non-suitability of the bread or the cooling and storage conditions, or both.

It is realised, of course, that a flour of high spore content will produce ropy bread more readily than a flour of low spore content if both are *subjected to exactly the same treatment during fermentation and baking of the dough and cooling and storage of the bread*. It is only under such conditions, however, that the magnitude of the spore content becomes of first importance.

As the initial spore content of the flour is not the main factor, external contamination of doughs with spores from dirty troughs and benches, although dangerous, is not likely to have a great influence except in extreme cases. None of the cases we have investigated has been due to this cause.

Our experiments on commercial lines have convinced us that with the ordinary run of flours the important factor is the rate of development of the bacteria in the final bread, and this depends upon:—(1) The fermentation of the dough; (2) the baking of the dough; (3) the cooling of the bread; (4) the storage of the bread.

The fourth factor was extensively studied by Watkins (1906), who confirmed the general opinion that the trouble occurs mainly in summer time, *i.e.* when climatic conditions are favourable to the development of the disease. Our work was intended to indicate those factors most helpful in retarding the spread of the disease in bread subjected to such favourable condition of storage. Hence we decided to confine ourselves to an investigation of the first three factors.

In this section of the investigation we were not concerned with the academic side of the question, but hoped to obtain some definite data which would be helpful

to the baking trade. In view of this, we did not base our decisions as to the degree of ropiness of the different loaves upon the results of sugar estimations (Lloyd and McCrea, 1918), or upon the changes in the catalytic activity (Bunzell and Forbes, 1930), but upon the intensity of those symptoms which commercial bakers know to be indicators of rope. These symptoms are the peculiar and distinctive odour (this is the earliest of these symptoms), patches of greyish to brown discoloration in the crumb, and stickiness of these discoloured patches and often of the rest of the crumb.

In all the following experiments, the final loaves were stored in a moist atmosphere at 27° C. for several days to assist the development of the disease, but the same conditions existed for all loaves.

(1) **FERMENTATION OF THE DOUGH.**—There are many commercial methods of bakery practice, and, therefore, it was thought best to study the following main factors:—(a) Duration of fermentation; (b) initial temperature of the dough; (c) percentage of yeast incorporated in the dough; (d) percentage of water used for doughing. All the following baking tests were performed in a well-designed and equipped bakery by an expert and experienced test baker. It should be remembered that in small test bakes a greater percentage of yeast must be used than in commercial practice with large doughs. This, of course, does not interfere with the comparative value of the tests.

(a) *Duration of Fermentation.*—It was noticed quite early in these investigations that loaves made from a flour of high spore content on a short system (3½ hours to oven) showed only a mild development of the disease after several days' storage in a warm, moist atmosphere, *i.e.* under conditions favourable to its development. As a much stronger development was expected, it appeared that a short fermentation tended to inhibit rope development. To confirm this point, two series of 2-lb. tin loaves were made from the same flour, but one series was made by a 3½-hour system, and the other series by a 7½-hour system. After appropriate storage, the loaves were cut into halves. As expected from the method of storage, all the loaves were ropy, but those made on the short system were less affected than the others. The long-system loaves had the stronger odour and the stickier crumb, which was discoloured; the crumb of the short system loaves showed no discoloration. This experiment was repeated on several occasions, and each time the long-system loaves became decidedly more ropy than those made on the short system. Both the loaves in Fig. 1 were made from the same flour, and were stored under identical conditions, but a 3½-hour system was used for loaf A, whilst loaf B was made by a 7½-hour system. It is seen that loaf B is considerably more ropy than loaf A. In the shorter system, about 1.8 per cent. of yeast was employed, and about 1.1 per cent. in the longer system. As stated previously, such large quantities of yeast could not be used in commercial practice, but the relative proportions would not be dissimilar.

As it is known that acidity will inhibit rope, it was thought that the advantage of one system over another might primarily be due to a greater alteration in the

hydrogen ion concentration. On investigation, very little difference in the acidities was found and this could not, therefore, account for the differences in the development of the disease.

It may be that the gradual conditioning of the gluten during the longer fermentation results in the formation of nitrogenous bodies which are easily assimilated by the organisms, whereas in short vigorous fermentations such compounds may not be formed to the same extent.

(b) *Initial Temperature of the Dough*.—Three 2-lb. tin loaves were made from a commercial flour on a  $7\frac{1}{2}$ -hour system. The temperature of the doughing water was varied for each dough, but otherwise they all received exactly the same treatment throughout the test. The initial temperatures of the doughs were 70° F., 80° F., and 88° F., respectively; under the conditions of the test 80° F. was the normal initial temperature. Fig. 2 shows the appearance of the cut loaves after several days' storage under conditions favourable to the growth of the organisms. The letters A, B and C correspond with the initial temperatures 70° F., 80° F., and 88° F., respectively. Loaf A was decidedly the worst of the three. It had a stronger smell than the other two, and the middle of the crumb was very soft and sticky and badly discoloured. There was not a great deal of difference between loaves B and C, but loaf C was slightly more ropy than the other. Other similar tests have confirmed the fact that a low initial temperature in the dough aids the development of the disease in the final bread. On reflection, this is not unexpected, as, naturally, in such instances the fermentation would not be so vigorous, although no differences in the pH of the loaves when made were observed.

(c) *Percentage of Yeast Incorporated in the Dough*.—A series of loaves was made from a commercial flour on a  $7\frac{1}{2}$ -hour system and, except that they contained different amounts of yeasts, all the doughs were similar, and received exactly the same treatment. The amounts of yeast employed were 0.5, 0.8, 1.1, 1.4 and 1.7 per cent., respectively; the normal quantity for a test bake under these conditions is 1.1 per cent. After the usual several days' storage under conditions favourable to the growth of the organisms, the loaves were cut, and they showed marked differences in the degree of development of the disease. The loaves containing the normal quantity of yeast (1.1 per cent.) had a decided smell and a somewhat sticky, but only faintly discoloured, crumb. The loaves containing the larger proportions of yeast were appreciably less affected (particularly those with 1.7 per cent. of yeast), while the loaves with the smaller than normal quantity of yeast were decidedly the worst. Fig. 3 shows the difference in the ropiness of three of these loaves; loaf A contained 0.5 per cent. of yeast, loaf B 1.1 per cent. (the normal quantity), and loaf C, 1.7 per cent. Again, further experiments confirmed these results. The various loaves of the series did not show any appreciable differences in pH value.

(d) *Percentage of Water used in Doughing*.—For this investigation a series of loaves was made and all the doughs were similar except in their water content.

The amounts of water used in making the doughs were 52, 55.5, 58, 62, and 64 per cent., respectively; the correct quantity for this flour was 58 per cent. Those loaves which had been made with less than the correct amount of water did not differ very much in degree of ropiness from the loaves with the normal quantity, but, if anything, the disease seemed slightly less pronounced in the former loaves. Those loaves made with more than the correct amount of water were more ropy than those with the normal quantity and, in the case of those loaves with 64 per cent. of water, the disease was very marked. These points are illustrated in Fig. 4, where loaves A, B and C were made with 52, 58 and 64 per cent. of water respectively. Slacker doughs, therefore, appear to assist in the development of the disease.

(2) BAKING OF THE DOUGH.—A large dough was made on a 7½-hour system, and subsequently scaled off into 2-lb. pieces. After these had been moulded and proved, they were placed in the oven, but, instead of their all receiving a normal baking of 45 minutes, they were taken from the oven in batches after 25, 45 and 65 minutes' baking, respectively. When the loaves were eventually cut after severe storage, those that had received a normal baking (45 minutes) possessed a ropy odour and exhibited a few discoloured patches; those that had been baked for only 25 minutes had a very soft, sticky and discoloured crumb, and smelt very strongly; those that had been baked for 65 minutes exhibited no symptoms of the disease save for a very faint ropy odour. Loaves A, B and C, in Fig. 5, had received 25, 45 and 65 minutes' baking respectively. This confirms the general view in the baking trade that, in times of doubt, the bread should be well baked.

(3) COOLING OF THE BREAD.—It is well known that both warmth and moisture exert a positive influence upon the development of ropiness. Obviously, therefore, any conditions which tend to retard the cooling of hot bread will hasten the appearance of the disease.

On several occasions half the loaves from a batch of bread were allowed to cool slowly in the bakehouse (temperature 24° C. approximately) overnight, while the others were cooled rapidly outside (temperature 12° C. approximately) for an hour, and then placed in the bakehouse. Subsequently all the loaves were placed in a warm, moist atmosphere in accordance with our usual procedure. After several days' storage, those loaves which had been rapidly cooled were appreciably less ropy than the others.

The rate of cooling of a loaf is affected by its size. A large loaf cools more slowly than a smaller one, and hence it is to be expected that, under identical storage conditions, large loaves will become more ropy than small ones. To test this point, a number of both 2-lb. tin and 4-lb. tin loaves were made from the same dough and were cooled and stored together. In every case the loaves of quartern size were more ropy than the 2-lb. loaves after our usual incubation.

The cooling of bread is considerably retarded if the loaves are wrapped soon after leaving the oven. A series of loaves was made from a large dough and, soon after they had left the oven, a number of them were wrapped. The loaves, wrapped

and unwrapped, were stored for several days and then cut. The wrapped loaves always showed a more marked development of the disease than did the unwrapped loaves. Of course, if the loaf is well cooled before wrapping, the trouble is not aggravated.

The pairs of ropy loaves obtained in the various tests described in this section were very similar in appearance to the two loaves of Fig. 1, and were, therefore, not reproduced.

CONCLUSIONS.—The rope spore content of flour, unless particularly high, is of little significance in outbreaks of rope in commercial practice. While it is helpful that flour of low spore content, or, at least, of not very high spore content, should be supplied by the miller, of far greater importance are the systems of fermentation, baking and bread cooling employed by the baker.

That the technique employed in the bakehouse can influence the incidence of the disease in the bread is known to the trade, but whether the importance of this factor has been sufficiently recognised is doubtful. Further, opinions differ as to the manner in which variations in bakery practice affect the development of ropiness. Thus, Wihlfahrt (*A Treatise on Baking*, 1927) states that when rope appears, the doughs should be made stiffer and at a relatively low temperature, whereas Bennion (*Breadmaking*, 1929) advises the use of freer doughs and a suitably high temperature.

Our own experiments have convinced us that when this trouble occurs, the following rules should be observed in fermenting the dough: (1) Avoid a long system of fermentation; (2) use a liberal quantity of yeast; (3) avoid low temperature in the dough; (4) avoid slack doughs. It is possible that attention to this and the following advice might permit the eradication of the trouble without the use of acid additions, such as vinegar and acid calcium phosphate, although this latter practice can usually be easily carried out and is effective.

Care should be taken that the dough is well baked, since an underbaked loaf is a medium particularly favourable to the growth of the organisms.

The bread should be cooled as rapidly as possible in a well-ventilated space and, if the loaves are to be wrapped, this operation should not be performed until they have been well cooled.

In connection with the cooling of the bread, it is as well to remember the effect of the size of the loaves. It is advisable, when trouble is threatened, to make no loaves larger than the 2-lb. size. Quartern loaves, especially if they are banked together, will only tend to increase the trouble. As a matter of fact, we found that one of the firms who responded to our appeal was experiencing bad trouble at a large institution solely from this cause. All the bread was made in quartern tin loaves which were banked together, so that the cooling was unduly prolonged.

Our findings do not mean that a knowledge of the spore contents of flours is of no value. To a miller such information will indicate whether his flour is more



than usually liable to produce ropy bread, and whether it is advisable to modify his wheat-cleaning procedure; to a baker it will indicate whether any trouble experienced is the result of his own baking procedure or whether he is handicapped by the possession of an abnormal flour.

What our results do prove, however, is that it is quite impossible to fix a universal safety limit for the number of rope spores in flour; the safety line depends entirely upon the methods used for making, baking, cooling and storage of the bread.

It should be remembered that rope is encountered in warm countries far more than in England, as, for instance, in South Africa, where one of us saw several instances recently. Under such climatic conditions all bread will become ropy very rapidly, and, were it not for the fact that bread is consumed quickly, the trouble would be even more prevalent.

Rope is often more severe with long extraction flours or wholemeals. It is true that such flours are often inclined to have fairly high spore contents, but, after our investigations, we should like to re-emphasise the findings of Lloyd, Clark and McCrea (1921) in this respect, namely, that the higher the extraction of the flour the better medium it is for the growth of *B. mesentericus*.

Finally, it is hoped that this work may be of value in settling many controversies in the baking trade. We have made the investigation as wide as possible, so that our findings may be of practical value to millers and bakers also, especially those working under difficult climatic conditions. We have drawn upon a fairly wide experience of both trades and have endeavoured to perform the work in a judicial spirit. We must admit that the trend of our conclusions is surprising, but we are confident of them after the systematic and careful investigation we have made.

**SUMMARY.**—A method of determining the number of rope spores in flour is described which gives satisfactorily consistent results.

The rope spore contents of various flours, some of which were producing ropy bread under commercial conditions, are reported. These results indicate that the rope spore content of a flour is of less importance in the development of rope in commercial bread than the technique adopted in the bakery.

Experiments are described which show that the following conditions tend to aggravate rope trouble in bread which has been stored in conditions favourable to the development of the disease:—Long systems of fermentation; low temperatures in the doughs; sparing use of the yeast; slack doughs; insufficient baking; conditions causing slow cooling of the bread, such as wrapping the bread while warm, making quartern loaves, banking loaves together.

Our thanks are due to many millers and bakers who have assisted us in this work, and also to Mr. G. Austin, who has carried out the various baking tests.

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# DISCUSSION.

Dr. KENT-JONES said that he would like to emphasise the fact that "ropiness" was a matter of some importance to the milling and baking trade, and that they were always having to face the difficulty of having to deal with infuriated bakers (whose bread had gone "ropy"), who blamed the millers, or with millers who wanted to know if their flour really was the cause of "ropiness." At the outset they had thought that it must be something to do with the number of spores. Other conditions being equal, the number of spores actually was the deciding factor, but in ordinary commercial circumstances other conditions were not equal, and fermentation, baking, etc., affected it. It had been shown that flour with quite a high spore content could be baked in such a way that it would give no trouble. Therefore, they had thought they would be doing a service to the baking trade if they could ascertain the type of fermentation which made the trouble develop. Of course, in hot countries bread was more inclined to go "ropy" than in England. On a visit to South Africa, he had been surprised to see how much bread actually became "ropy"; practically all the bread would become "ropy," but for the fact that it was eaten before the trouble arose. They had tried, in this paper, to point out the conditions which would help those people who encountered the trouble. The loaves used in the tests had been purposely allowed to become "ropy"; they had been used as a standard to see what type made the trouble worse and what type retarded "ropiness."

Mr. R. L. COLLETT asked whether the authors had been able to produce "ropiness" artificially by inoculating flour or dough from a pure culture.

Mr. McLACHLAN asked, in connection with the authors' statement that the spores could not be killed by heat, whether they had tried steam pressure. Often when dry heat failed to kill, steam succeeded. Also, since malt extract was being increasingly added to bread, he asked whether bread containing malt extract tended to become more "ropy" than ordinary bread.

Mr. D. M. FREELAND enquired whether "ropiness" was liable to occur in cake.

Mr. AMOS, replying, said they had actually reproduced "rope" with pure cultures. They had inoculated doughs in marked positions with *Bacillus mesentericus vulgatus*, and "rope" had developed in those marked positions long before it had anywhere else. They had also sterilised tubes of bread and inoculated these. It was rather difficult to say whether Scotch bread would be more or less liable to "ropiness." The fermentation was slower, which would tend to make

the trouble worse, but, on the other hand, Scotch bread was decidedly more acid than English bread, and he thought that would tend to retard development. He had never yet met with a case of "rope" in cake. He believed it had been mentioned in the literature that a certain amount of acid developing from the fruit tended to inhibit "rope." He thought it quite probable that if one could sterilise long enough, dry heat would eventually kill the spores, but they had found it too long a process to use in their tests. They had made experiments on the effect of malt extract, and in some cases the trouble became worse, whilst in others it was not so bad. It seemed to depend on the quality and properties of the malt extract used.

Dr. KENT-JONES said that he had been asked a question as to the difference in this respect between white bread and brown bread. The spore contents of both white flours and wholemeals were recorded in the paper. The nature of the proteins, etc., in brown bread assisted the development of the disease.

## The Denigès-Oliver Test for Morphine.

BY F. BAMFORD.

(Read at the Meeting, March 4, 1931.)

THE Denigès-Oliver test for morphine and some of its derivatives has been known for some years, but it is doubtful whether it has attracted the attention which it deserves. It was described by Denigès in *Compt. rend.*, 151, 1062; *Répert. de Pharm.*, 1911, 10; *Apoth. Zeit.*, 1911, 66; and *Pharm. Zeit.*, 1911, 106; and in a slightly modified form by Oliver in *The Medical Chronicle*, 1914 (July), and *The Lancet*, 1914, II, 244.

The test consists in the partial oxidation of morphine by hydrogen peroxide in presence of ammonia and copper, with the formation of a red colour. Denigès used copper sulphate solution, and Oliver a piece of metallic copper.

Conflicting statements have been made as to the applicability of the test to derivatives of morphine. Peterson, Haines and Webster (*Legal Medicine and Toxicology*, 2nd Ed., Vol. II, p. 534) state, contradicting Oliver himself, that heroin gives a reaction similar to morphine. Webster also states that a red colour is obtained with dionin, and an orange colour with apomorphine. Our experience does not confirm either of these statements.

The test is used for routine work in this laboratory as confirmation of the Marquis test, and we find that a red colour is produced with morphine and its acid esters, as well as with apomorphine, but that no colour is obtained with codein or dionin.

It is not easy to say which is the preferable form of the test—that of Denigès or that of Oliver. In ordinary qualitative tests for the identification of narcotic

drugs, and also, as will appear, in quantitative work, the Denigès method is more convenient; but in testing residues obtained from viscera or excreta by the Stas-Otto process of extraction, probably better results are obtained with metallic copper, since the red colour develops on the surface of the copper and a coloured streak can frequently be very clearly seen, whereas there might be doubt about a pink colour dispersed throughout the liquid. The copper should be a piece of thin wire and should rest in the liquid. (Oliver recommends that the liquid should be stirred with the wire.)

The test is, in our opinion, the most reliable of the colour reactions of morphine and heroin, although it is less sensitive than the Marquis and Fröhde tests. Tests, like that of Husemann, which depend on the production of reddish-brown colours with strong acids are to be interpreted with caution when the substance under examination has been extracted from viscera or excreta, and even Fröhde's reaction, excellent as it is when the morphine is pure, is liable to be misconstrued if impurities capable of reducing the molybdate are present. In testing samples of alleged narcotic drugs, and also extracts obtained by the Stas-Otto process, we use first the Marquis reagent (formaldehyde and sulphuric acid), watching the colour change through red-violet to bluish-violet and, before affirming the presence of morphine or one of its esters, we always confirm this reaction by the Denigès-Oliver test.

In the course of experiments on the possibility of applying the test for quantitative purposes it has been found that the stability of the colour depends on the quantity of hydrogen peroxide used. It would appear that the red substance produced is an intermediate product in the oxidation of morphine, and that the oxidation readily goes beyond this stage in presence of excess of oxygen.

In fact, with a small amount of morphine and a large excess of hydrogen peroxide the colour fades so rapidly that there may be a doubt whether it has been seen at all.

Heroin resists the further oxidation better than morphine, and heroin was, therefore, chosen for the preliminary experiments to determine the optimum conditions of the reaction. The colour obtained is almost identical in shade with Congo red, but the tinctorial strength of the dyestuff is about twenty times that obtained from an equal weight of heroin. A solution of Congo red containing 5 parts per million was used as a fixed standard of comparison in order to find the best conditions for the reaction, and it was found that for 1 mgrm. of heroin hydrochloride in 5 c.c. of water the maximum colour intensity was quickly attained by adding 4 c.c. of a solution of hydrogen peroxide containing 2 grms. per litre (*i.e.* one yielding about two-thirds of its volume of oxygen). The quantity of copper should be minute, one drop of a solution containing about 1 grm. per litre being sufficient. The amount of ammonia appears to have little effect, two, three, or four drops of a concentrated solution being a convenient quantity. Such a mixture, diluted to 10 c.c., yields a colour equal in intensity to a Congo red solution made by dissolving 5.3 parts of the dyestuff (Kahlbaum-Schering) in

1,000,000 parts of water. The maximum colour intensity is attained in about five minutes, and does not fade within half-an-hour.

This solution, containing 1 part of heroin hydrochloride in 10,000, may be diluted to 1 in 10, and the colour is still perceptible when compared with pure water in a Duboscq colorimeter with a depth of liquid of 50 mm., showing that the test is sufficiently sensitive to detect 1 part of heroin hydrochloride in 50,000 parts of water (100,000 when the solution has been diluted after the addition of the reagents). As a qualitative test, 0.02 mgrm. of heroin in 0.1 c.c. of water, treated with the appropriate quantities of reagents, gives a colour which is perceptible on a white tile.

For quantitative purposes a solution containing 1 part of heroin hydrochloride in 5000 of water, diluted to 10,000 after the addition of the reagents, has been found most convenient, although it is possible to use a more dilute standard if desired. The standard solution was prepared from pure crystallised heroin base (m.pt.,  $171^{\circ}\text{C}.$ ), dissolved in the theoretical quantity of  $N/10$  hydrochloric acid and afterwards diluted.\*

The colour obtained from heroin was found to obey Beer's law within the limits usually obtaining in colorimetric work; *i.e.* the intensity was proportional to the quantity of heroin present when the two solutions compared did not differ by more than 20 per cent. in concentration. Thus, taking 1 part in 5000 as the standard, accurate readings were obtained with solutions containing 0.8 or 1.2 part in 5000, but not with 0.5 part or 2 parts.

The chief application of the colorimetric method in this laboratory is for the determination of the proportion of heroin in the adulterated samples of narcotics sold illicitly, and here the commonest adulterant, apart from inorganic salts, is quinine. Now quinine, if present in more than small proportions, interferes with the sensitiveness of the test, and it was, therefore, necessary to devise a method of separating quinine from heroin. For this purpose advantage may be taken of the relative insolubility of (a) the sulphate, or (b) the tartrate of quinine.

The addition of a strong solution of Rochelle salt precipitates quinine tartrate in fine crystals in a moderately short time, whilst saturated sodium sulphate solution causes less complete separation, even when allowed to stand for 24 hours or more. Nevertheless, if quinine is the only organic adulterant, the latter method gives a convenient means of separating the two alkaloids without recourse to immiscible solvents, which must be used if the tartrate method is employed, since tartaric acid also interferes with the delicacy of the test.

*Examples.*—One c.c. of a 1 per cent. solution of heroin hydrochloride and 1 c.c. of a 1 per cent. solution of quinine hydrochloride were mixed with saturated sodium sulphate solution so as to make the volume 20 c.c., and allowed to stand for 48 hours. The greater part of the quinine separated in fine needles; and

\* Attempts had been made to obtain pure heroin hydrochloride by taking advantage of its solubility in dry chloroform, filtering off inorganic matter, and diluting with benzene, but the results were unsatisfactory.

although the solution still showed the fluorescence characteristic of quinine, the proportion of that alkaloid still present was not sufficient to mar the determination. Two c.c. of the filtrate (containing 1 mgrm. of heroin hydrochloride) was found to be exactly equivalent to 1 mgrm. of the pure substance when treated as above described, the final volume being made up to 10 c.c., and the readings of the colorimeter being made at 15 mm. This method, however, is useless if other unknown organic impurities are present in addition to quinine. In such circumstances extraction with immiscible solvents is essential, and there is no reason, therefore, why the more rapid and complete precipitation of the quinine as tartrate should not be employed.\*

*Example.*—One c.c. of a 1 per cent. solution of quinine hydrochloride and 1 c.c. of a 1 per cent. solution of heroin hydrochloride were mixed with sufficient 50 per cent. solution of Rochelle salt to make the volume 20 c.c. After about three hours the quinine tartrate was filtered off through a dry filter, and 10 c.c. of the filtrate were transferred to a separating funnel and shaken out three times with a mixture of ethyl acetate and ether, using about 5 c.c. each time.

The collected extracts were returned to the separator and washed once by shaking with distilled water. The extract was then evaporated to dryness, and the residue was taken up in a few c.c. of dilute sulphuric acid. This solution was diluted again to 20 c.c., and, of this dilution, 4 c.c. were found to agree exactly with 1 mgrm. of heroin hydrochloride dissolved in water.

An attempt was made to avoid the shaking-out process here also, by precipitating the tartrate with saturated calcium chloride solution, separating the insoluble matter by means of the centrifuge, and applying the colorimetric method to the clear supernatant liquid; but the result was low, either because the tartrate was not completely removed, or because the presence of large amounts of chlorides is inimical to the reaction.

The work will be continued to find the exact conditions for the determination of morphine itself and the possible application of the process to the assay of opium.

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\* Lactose, a common adulterant, does not, however, cause any interference.

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The principle of the method is, briefly, that there exists a fairly wide range of acid concentration within which chromic acid rapidly and completely oxidises bromides to bromine whilst leaving chlorides untouched; the best working strength of acid is 8 to 9 *N* sulphuric acid, and, in practice, this is most readily attained by dissolving the sample in water and adding a third of its bulk of concentrated sulphuric acid. The apparatus used is that described for the solution of high chromium steels for purposes of sulphur determination (B. S. Evans, *ANALYST*, 1929, 54, 286), of which a diagram is included.

**METHOD.**—Details of the process are as follows:—The leading tube of the apparatus is passed through one hole of the double-bored rubber stopper of a flask containing a measured excess of standard arsenious oxide solution together with several grms. of sodium bicarbonate and about 200 c.c. of water; the other hole of the stopper carries a short tube bent at right angles and connected with a filter pump, so that a stream of air can be drawn through both flasks. The empty flask is detached from the apparatus and the sample added in the form of a solution in a roughly measured bulk of water, one-third of its volume of concentrated sulphuric acid is then run in, and the whole is completely cooled; about 5 grms. of solid chromic acid are then quickly added, the neck, if necessary, rinsed down with a little dilute (1:3) sulphuric acid, and the flask at once re-attached to the apparatus. A steady stream of air bubbles is then drawn through the two flasks for an hour, the tap of the apparatus being left open; at the end of this time the absorption flask is disconnected and, after rinsing down the sides and the tube with water, the excess arsenious oxide is titrated with standard iodine. If *N*/10 solutions are used, the difference between this figure and the titration figure of the arsenious oxide originally added, multiplied by 0.0080, gives the grms. of bromine present in the sample taken. With a view to keeping the air stream steady it is desirable that the connection with the pump should run through a T-piece, the side arm of which is connected with the outlet tube of a wash bottle containing a suitable depth of water, so that, whatever the variation of the pump, a steady head of pressure is maintained in the apparatus, the excess pressure being shunted through the wash bottle.

The following results were obtained with solutions of sodium chloride to which varying amounts of bromide had been added, the composition of the samples being unknown to the operator at the time of making the determinations:—

Sodium chloride present. Grms.	Potassium bromide added. Grm.	Titration, <i>N</i> /10 $\text{As}_2\text{O}_3$ , c.c.	Potassium bromide found. Grm.	Potassium bromide added. Per Cent.	Potassium bromide found. Per Cent.
3.0	0.0330	20.00–17.00 = 3.00	0.0357	1.10	1.19
3.0	0.0690	20.00–14.00 = 5.90	0.0702	2.30	2.34
3.0	0.0060	20.00–19.45 = 0.55	0.0065	0.20	0.22
3.0	0.1340	20.00–8.80 = 11.20	0.1333	4.47	4.44
3.0	Nil	20.00–20.00 = Nil	Nil	—	—
3.0	0.0730	20.00–13.75 = 6.25	0.0744	2.43	2.48



The process thus gives very fair results when the concentration of sodium chloride does not exceed 3 grms. per (say) 200 c.c. of liquid in the evolution flask; if, however, it becomes necessary to take a larger sample, somewhat high results are obtained; thus experiments carried out varying the amount of chloride gave the following results:—

Sodium chloride taken. Grms.	Potassium bromide added. Grm.	Titration, $N/10 \text{ As}_2\text{O}_3$ , c.c.	Potassium bromide found. Grm.
Nil	0.1000	20.00–11.70 = 8.30	0.0988
5.0	0.1000	20.00–11.50 = 8.50	0.1011
10.0	0.1000	20.00–11.40 = 8.60	0.1023

CHLORIDES CONTAINING SMALL AMOUNTS OF BROMIDE.—In dealing with small percentages of bromides in chlorides, therefore, it is better to proceed as follows:—The process is carried out as usual, except that the absorption flask is replaced by a tube containing about 40 c.c. of 0.5 per cent. sodium hydroxide solution. At the end of the hour's bubbling the tube is detached, one or two drops of saturated solution of sulphur dioxide are added, and the contents are neutralised to methyl orange by addition of dilute (1:3) sulphuric acid, and roughly measured; one-third of its bulk of concentrated sulphuric acid is added, and it is transferred to a clean evolution flask, the leading tube of which dips into standard arsenious oxide solution as in the original process; the solution is rinsed in with dilute (1:3) sulphuric acid, chromic acid is added, and the process finished as usual. This method gave the following result:—

Sodium chloride taken. Grms.	Potassium bromide added. Grm.	Titration $N/100 \text{ As}_2\text{O}_3$ , c.c.	Potassium bromide found. Grm.	Potassium bromide	
				added. Per Cent.	found. Per Cent.
10.0	0.0020	40.00–38.50 = 1.50	0.0018	0.020	0.018

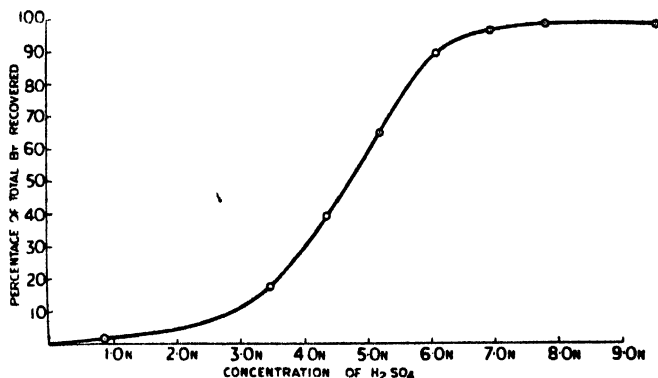
It would seem, therefore, that, by thus effecting the separation in two stages, a determination of minute amounts of bromide in chloride of considerable accuracy can be obtained.

Some experiments were undertaken to establish the relationship of the acid concentration to the efficiency of oxidation of the bromide. In each case 0.100 gm. of potassium bromide was taken and 5 grms. of chromic acid; no chloride was added and the total volume of the solution was 110 c.c.; the acidity of the mixture was arrived at by titration of a 1:3 mixture of sulphuric acid and water and using known volumes of the latter suitably diluted, the assumption being made that volume change on dilution was negligible. After the addition of the chromic acid, air was passed through the mixture, and through the arsenite and bicarbonate mixture, which was subsequently titrated exactly as described in the process. The results were as follows:—

Potassium bromide taken. Grms.	Acid strength.	Titration, $N/10 \text{ As}_2\text{O}_3$ , c.c.	Percentage of bromide recovered.
0.100	0.86 <i>N</i>	20.00-19.85 = 0.15	1.8
0.100	3.48 <i>N</i>	20.00-18.55 = 1.45	17.3
0.100	4.33 <i>N</i>	20.00-16.75 = 3.25	38.7
0.100	5.19 <i>N</i>	20.00-14.60 = 5.40	64.3
0.100	6.06 <i>N</i>	20.00-12.45 = 7.55	89.9
0.100	6.92 <i>N</i>	20.00-11.90 = 8.10	96.4
0.100	7.79 <i>N</i>	20.00-11.70 = 8.30	98.8
0.100	9.52 <i>N</i>	20.00-11.70 = 8.30	98.8

The last acid strength mentioned (9.52 *N*) corresponded with undiluted 1:3 sulphuric acid.

These results plotted in the accompanying curve show clearly that above an acid strength of approximately 7.5 *N*, oxidation is complete.



An attempt made to construct a similar curve for oxidation of chlorides failed, because at ordinary temperatures and with manageable concentration of acid the oxidation was practically nil, thus:

Sodium chloride taken. Grm.	Acid strength.	Titration, $N/10 \text{ As}_2\text{O}_3$ , c.c.	Percentage of chloride recovered.
0.100	15.84 <i>N</i>	30.00-29.90 = 0.10	0.6

These figures show that, using an acid concentration of about 8 to 9 *N*, separation of bromides from chlorides is practically complete if the chloride concentration is not too high, and the double treatment, by ensuring that only traces of chloride are present in the actual determination, allows separation in any proportion.

Before trying the above process, many attempts were made to effect a separation using bromate, iodate, permanganate and chlorate as oxidising agents with sulphuric, hydrochloric, nitric and phosphoric acids, but success was rare and uncertain.

## The Rapid Determination of Small Quantities of Lead in Calcium Phosphate.

By JOHN RALPH NICHOLLS, B.Sc., F.I.C.

LEAD may be determined colorimetrically as sulphide either in acid or in alkaline solution. The latter is much the more delicate, and most methods employ, eventually, an ammoniacal solution. Such an ammoniacal solution must be free from precipitate or turbidity and, in the case of calcium phosphate, means must be found of achieving this.

The process described by Francis, Harvey and Buchan (*ANALYST*, 1929, **54**, 725) for the determination of lead in biological material may be shortened for the particular case of calcium phosphate, but even then it takes considerable time. The quantitative limit test for lead of the British Pharmacopoeia is very rapid in application, but none of the artifices there employed is capable of producing a clear ammoniacal solution with calcium phosphate. If, however, the precipitation of the phosphate could be prevented, this process might be employed.

It has long been known that citric acid and citrates exert a solvent action on the various phosphates of calcium, and Terreil (*Bull. Soc. Chim.*, 1881, **35**, 548) showed that a solution containing 100 grms. of citric acid neutralised by ammonia dissolved 7 grms. of calcium phosphate.

The calcium phosphates of commerce are very variable in composition and differ considerably in their solubility. Most samples are more or less readily soluble in citric acid solutions, but some do not dissolve completely even on prolonged boiling. All, however, dissolve in hydrochloric acid, and the addition of 10 to 15 grms. of citric acid for each gm. of calcium phosphate dissolved is sufficient to prevent precipitation on subsequent addition of ammonia. The latter must, however, be added gradually as heat is evolved and, since strong ammonia should be used to keep the volume as small as convenient, local precipitation may occur. It is advisable, therefore, to replace the citric acid by ammonium citrate, which may conveniently be prepared by the neutralisation of citric acid. A stock solution may be made by dissolving 400 grms. of lead-free citric acid in water, gradually adding about 340 ml. of concentrated ammonia and diluting to 1 litre.

Applying the procedure of the British Pharmacopoeia lead test, the method may be summarised as follows:—

Dissolve 1 gm. of calcium phosphate in 10 to 15 ml. of 2 *N* hydrochloric acid, and add about 30 ml. of a solution containing 45 to 50 per cent. of ammonium citrate. Treat the solution with 2 ml. of 10 per cent. potassium cyanide solution made faintly alkaline with ammonia, and add two drops of sodium sulphide

(10 per cent. solution made from colourless crystals). Compare the colour produced with standards containing similar quantities of reagents to which known amounts of dilute solution of lead (0.001 per cent.) have been added. One ml. of 0.001 per cent. lead solution is equivalent to 10 parts per million of lead.

If the calcium phosphate contains more than traces of iron, this should be reduced by sodium thiosulphate according to well-known methods (*e.g.* Wilkie, *J. Soc. Chem. Ind.*, 1909, 28, 636) before adding the potassium cyanide and ammonia.

Where a higher degree of accuracy is required with samples containing less than about twenty parts per million of lead, take 10 grms. of calcium phosphate and make an ammoniacal solution as described with ten times the volumes of reagents given above. Treat with hydrogen sulphide and, when the precipitate has settled, filter the solution through a small acid-washed filter paper. Transfer the paper and precipitate to a silica beaker, destroy by wet combustion with 2 ml. of sulphuric acid and about 10 ml. of nitric acid, and evaporate to dryness. Dissolve the lead sulphate in hot ammonium acetate solution and determine the lead in the usual way. A blank determination on the reagents must be made.

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## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### THE DETERMINATION OF ALCOHOL IN DILUTE SOLUTION BY MEANS OF ACID DICHROMATE, COMPARED WITH THE SPECIFIC GRAVITY METHOD.

ALTHOUGH the specific gravity of dilute alcohol, if accurately determined, say, to the fifth place of decimals, provides a quick and reliable method for estimating small alcoholic content, yet frequently one requires a confirmatory method equally quick and reliable. Moreover, specific gravity tables of methyl, isopropyl and alcohols other than ethyl alcohol are not always available, nor may the volume of spirit distillate be sufficient for its accurate specific gravity determination by bottle. In this laboratory, for example, it has been necessary to determine so small a quantity as (1) 0.1 per cent. of ethyl acetate in acetic acid, (2) 0.1 per cent. of ethyl lactate in lactic acid, (3) 0.9 per cent. of ethyl alcohol in ethyl acetate, and (4) 0.25 per cent. of alcohol in ether, and we have found that, after distillation with excess alkali as in (1) and (2), or extraction with brine, as in (3) and (4), the acid dichromate method (*cf.* ANALYST, 1929, 54, 134) is as reliable as it is convenient. It is, of course, necessary to be certain that no organic matter save alcohol is present in the distillate. The necessity for the presence of a large excess of sulphuric acid

to complete the reaction  $C_2H_5OH + O_2 = CH_3COOH + H_2O$  at room temperature, has been frequently emphasised. The mixture we have found satisfactory consists of 90 c.c. of *N* potassium dichromate solution and 600 c.c. of concentrated sulphuric acid diluted to 1 litre with water. A comparison of results obtained with the specific gravity bottle and by "oxidation" may be of interest.

#### Ethyl Alcohol.

By means of the specific gravity bottle.	By oxidation with dichromate and sulphuric acid.
Per cent.	Per cent.
0.160	0.156
0.98	0.96
1.00	1.00
0.502	0.504

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#### POISONING BY ACETIC ACID.

SEVERAL fatal cases of poisoning by acetic acid have recently occurred in the Federated Malay States, and, as *post mortem* appearances after poisoning by acetic acid have not been described in the literature on this subject, the following two cases are described:

(a) At 3.0 a.m., February 11th, 1930, a Chinese shopkeeper, aged 40 years, was found by his wife in considerable distress. He was perfectly well the previous evening, when his last meal, of rice and curry, had been taken at about 7.0 p.m. He was in considerable pain, but he was conscious and there was no vomiting. He was admitted to hospital at 5.0 a.m., and given a few ounces of milk containing half-an-ounce of chalk. He died shortly after admission, and was conscious almost to the last. He was said to have swallowed about a pint of concentrated acetic acid just before 3.0 a.m.

*Post-mortem* examination showed no external signs of injury. The eyes were semi-dilated. The lips, tongue, mouth, trachea, and oesophagus were deeply corroded, and the mucous membrane was destroyed in places. The whole of the upper gastro-intestinal tract showed extensive corrosion, with intensive blackening of tissue. The contents of the stomach and gut consisted of a thick black fluid, resembling tar in appearance.

The mucous membrane of the stomach was completely destroyed—only small and narrow necrotic islands remained recognisable, while the stomach wall itself was perforated. There was advanced necrosis of the submucous tissue, with no recognisable elements. Fragmentation of fibres of the muscular coat was observed. The appearance of the respiratory and other internal organs was normal.

*Chemical Analysis.*—The oesophagus, the stomach with its contents, and a portion of the small intestine were received for chemical examination. They were received in one jar, preserved in 96 per cent. alcohol (rectified spirit).

The whole reacted acid to litmus; it was accordingly minced, and half of the mixture was diluted with distilled water and distilled with steam. The other half was reserved for further examination, if required.

The distillation was continued until the distillate no longer reacted acid to litmus, and then, after mixing, an aliquot portion of the distillate was titrated with  $N/10$  sodium hydroxide solution, with phenolphthalein as indicator.

A further portion of the distillate was made just alkaline with dilute sodium hydroxide, and evaporated to dryness on a water-bath. The presence of acetate in the residue was established by the formation of ethyl acetate with ethyl alcohol and strong sulphuric acid, and the absence of formic acid was proved by failure to reduce ammoniacal silver nitrate.

The absence of hydrochloric acid, the only other likely volatile acid, was proved. The total amount of acetic acid found in the stomach and small intestine was 5.6 grms.

(b) In the forenoon of December 24th, 1929, a male cook (Malabar), aged 25 years, is said to have told his friends that he had taken a purgative. He was quite normal until 6.0 p.m. the same day, soon after which he appeared to be drunk. He began to shout, jumped out of the window of his room and ran along the road for about ninety yards, where he lay down. Here he continued to shout and became even more excited. His friends carried him back to his room, where he lay on his bed, still raving, and shouting.

About 9.0 p.m. he quietened down, and at about midnight he was found lying unconscious on the floor of his room with a brown froth exuding from his lips. He had never been in a condition to say if he had swallowed anything. He died six hours after admission to hospital, not having recovered consciousness. There had been no vomiting or purging. *Post-mortem* examination showed no signs of recent external injury.

The mouth, gullet and stomach showed extensive corrosion and a very characteristic thickening and pronounced blackening of the stomach wall. The serous coat was brownish-black in appearance with paler yellow areas, giving a mottled appearance, which was uniform over the whole of the serous coat. On cutting through the stomach wall, the edges of the cut were reverted. The whole of the mucous membrane was black, and thrown into large rugose folds. It was very thickened (up to two inches in places) and oedematous to the touch. The detail of the mucous membrane was entirely lost to the naked eye. The respiratory system and other internal organs showed no abnormal appearance.

On chemical examination, as described in (a), 3.38 grms. of free acetic acid were found in the stomach and contents.

In this case, a bottle of strong acetic acid and a bottle of "Milton," belonging to the deceased's master (a European), were found on a sideboard near the wine cupboard. It is believed that, while in a drunken condition, the deceased swallowed some of the acetic acid in mistake for whisky or brandy. The quantity taken is unknown.

Access to strong acetic acid is readily obtained throughout Malaya, as it is extensively used on rubber estates for the coagulation of latex.

R. W. BLAIR.

INSTITUTE FOR MEDICAL RESEARCH,  
KUALA LUMPUR,  
FEDERATED MALAY STATES.

## Official Appointment.

THE Minister of Health has approved the appointment of:

F. W. HOYLAND, as Additional Public Analyst for the County Borough of Bournemouth, being terminated, he having left the employment of Mr. R. A. Cripps.

## Notes from the Reports of Public Analysts.

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF BIRMINGHAM.

#### ANNUAL REPORT OF THE CITY ANALYST FOR THE YEAR 1930.

OF the 4912 samples taken under the Sale of Food and Drugs Act, 188 were adulterated. The number of samples represented a purchase of 500 per 100,000 of population.

**MILK.**—Much time and labour have been expended by some apologists in trying to show that a very large proportion of genuine milk is actually below the presumptive limits of the Sale of Milk Regulations, and also to prove that a herd of cows is so unreliable in its habits that the quality of its milk may vary enormously from day to day. If some of the conclusions to which these workers have come are to be accepted as correct, it would be practically impossible ever to convict a farmer for selling adulterated milk. The experience of practically all Public Analysts has been that very little milk obtained from a herd of healthy cows falls below the limits, and most of it is considerably over the limits. In any case, so far as Birmingham and many other districts are concerned, if a sample is found to be deficient in solids, very exhaustive enquiries are made, and generally a large number of further samples is procured before taking any legal action.

**Nitrates in Milk.**—All doubtful samples are examined for nitrates, which, if present, constitute a proof that water has been added. Nitrates have never yet been found in genuine milk.

**LABELLING OF JAM.**—In my opinion there should be at least three qualities of jam, and the first quality should be made from fruit and sugar only, without any addition whatever. The present first and second quality jam might be called second and third quality respectively, and any additions of whatever kind should be stated on the label. Apart from this, there should be some means of distinguishing between jam made from fresh fruit and that made from imported pulp preserved with sulphur dioxide.

**CREAM.**—Thirty-eight samples of fresh cream in cartons, four samples of tinned cream, and four of clotted cream were submitted during the year. The fat content of the fresh cream varied from 31·5 to 63 per cent., and averaged 53 per cent. The fat content of the tinned cream varied from 20·5 to 26 per cent., with an average of 22·5 per cent. The purchaser of tinned cream, therefore, gets, on an average,

less than half the amount of fat that he gets if he buys cream in a fresh state. The manufacturers of sterilised cream allege that it is impossible to prepare an article containing more than 25 per cent. of fat. If this be the case, it is only right that the purchasers should be made aware of it by a declaration on the label. This is actually done in some cases.

One sample of tinned cream bore on the label the words "Butter fat, 23 per cent. Total milk solids, 30 per cent. A highly concentrated dairy cream." This label was reported as being a false one, as, although it actually contained the amount of fat and solids mentioned, there was no justification for calling it a rich dairy cream, which should contain at least 50 per cent. of fat. The packers were approached and agreed to have new labels printed, on which these words should not appear.

TEA.—Four of 17 samples had false labels. One bore the words "Contains the maximum of theine with the minimum of tannin." Analysis showed that both constituents were present in about the usual proportions, and that the statements were untrue and misleading. The packers were communicated with and undertook to have new cartons printed.

Another sample was described as being "free from crude tannin" and "practically tanninless," whereas the percentage of tannin was 14 per cent. The packers agreed to omit the reference to tannin on their labels.

PREPARED HONEY.—A sample of prepared honey, sold under a trade name, had on the label the statement: "Made from honey but better than honey." As a matter of fact, it was found to contain a large proportion of commercial invert sugar, which, although consisting of the same kinds of sugars as honey, cannot be described as honey any more than a patent spirit can be described as whisky. The manufacturers were communicated with and gave an undertaking to sell the product in future labelled with the declaration, "This is sold as a mixture of honey and invert sugar." In fairness to the makers it should be stated that the product was perfectly wholesome and contained vitamin *D* in the form of ostelin. This, of course, is not present in ordinary honey, and the addition increases its value as a foodstuff.

BORAX HONEY.—This article should consist, according to the B.P., of 10 per cent. of borax, 5 per cent. of glycerin, and 85 per cent. of honey. It is used in children's throat and mouth affections. The informal sample contained no honey, and consisted of a mixture of 32 per cent. of borax and 68 per cent. of glycerin. The formal sample was of very similar composition, containing 40 per cent. of borax and 60 per cent. of glycerin. Honey was again absent. The vendor was prosecuted and fined 10s.

H. H. BAGNALL.

## CITY AND COUNTY OF BRISTOL.

### REPORT OF THE PUBLIC ANALYST AND BACTERIOLOGIST FOR YEAR 1930.

FIFTY-SIX of the 1400 samples of foods and drugs were adulterated.

MILK.—One sample had a pink colour, which was found to be due to eosin (0.00007 per cent.), and was traced to accidental contamination of the milk with a particle of dry red ink. Formaldehyde (0.004 per cent.) was found in one sample of milk, and in one sample of skim milk (0.006 per cent.).



**UNFERMENTED CORDIALS.**—Five samples of so-called “non-alcoholic British wines” gave the following results on analysis:

Description.	Ginger.	Orange.	Raisin.	Black currant.	Ruby.
Sp. gr. . . . .	—	1101.1	1083.3	1177.6	1109.6
Alcohol, by wt., per cent.	0.16	0.16	0.58	0.21	0.83
Alcohol, by vol., per cent.	0.20	0.20	0.73	0.26	1.0
Per cent. proof spirit	0.35	0.35	1.28	0.46	1.74
Total solids, per cent.	—	24.9	22.4	44.3	29.8
Ash, per cent. . .	—	0.072	0.072	0.106	0.03

**CANE SUGAR CONTAINING FORMIC ACID.**—Two samples taken at the port were alleged to contain formic acid as a preservative. Portions of the samples were acidified with tartaric acid and submitted to steam distillation. Formic acid was identified in the distillate and was determined by reduction of mercuric chloride under a reflux condenser. Parallel experiments on blank samples and on samples containing a known amount of formic acid confirmed the analysis. I have been unable to find any account of the physiological action of this preservative, and am therefore unable to say whether its presence is in any way dangerous.

E. RUSSELL.

## CITY OF LEICESTER.

### REPORT OF THE CITY ANALYST FOR THE YEAR 1930.

THE total number of samples examined was 2121, of which 1519 were foods and drugs and 264 were milks for bacteriological examination.

**BACTERIOLOGICAL STANDARDS FOR BOTTLED NEW MILK AND STERILISED MILK.**—The standard provisionally adopted for bottled new milk is that it must have a count not exceeding 1,000,000 bacteria per c.c., and must not contain *B. coli* in less than 0.01 c.c. If a sample fails in either respect, it is not passed.

Similarly, for sterilised milk a maximum count of 1000 per c.c. is adopted, and *B. coli* must not be present in 1 c.c. or less.

**CLEAR MINTS.**—Normally, the mints are made from granulated sugar and liquid glucose, and contain a small proportion of sulphur dioxide derived from the glucose. The maximum amount of sulphur dioxide introduced by the raw materials composing the mints, assuming 40 per cent. of glucose and 60 per cent. of sugar, would be 220 parts per million. In the boiling a good deal of this sulphur dioxide is lost; several series of laboratory tests have shown that usually not more than 25 per cent. of the original sulphur dioxide is retained as such in the final product. That is, mints containing 40 per cent. of glucose containing originally 450 parts per million of sulphur dioxide would, if otherwise untreated, contain not more than about 55 parts per million of sulphur dioxide, including that derived from the sucrose.

The rather unnatural whiteness of several brands on the market led to the suspicion that artificial bleach was being used, and samples of these brands were, in fact, found to contain excessive quantities of sulphur dioxide.

This undesirable and unnecessary practice of adding chemical bleach appeared to have obtained a great hold upon manufacturers, since one firm was found actually adding it to green-coloured lime drops, where, presumably, it would ultimately decolorise the green. One firm was found to be carefully adding to every 100 lbs. of sugar mixture one ounce of a strong solution of sodium bisulphite saturated with sulphur dioxide from a bottle labelled “Not to be used in Confectionery.”

Proceedings were instituted against the worst offenders and convictions obtained. Batches of samples taken subsequently showed that the haphazard use of bleach had stopped, but that it was still being used in carefully regulated quantity.

F. C. BULLOCK.

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### ORANGE AND QUININE WINE SOLD WITHOUT A LICENCE.

ON July 30, a druggist was charged at the Gateshead County Police Court with having sold a bottle of orange and quinine wine, without a licence, contrary to Sec. 65 of the Licensing Act, 1910.

Mr. C. J. H. Stock, the County Analyst, said that the wine contained 22·23 per cent. of proof spirit, 60·08 per cent. of additional water, 17·65 per cent. of extractives, and 0·228 grain of quinine hydrochloride per 100 ml.

In cross-examination, Mr. Stock did not agree that orange and quinine wine was a medicated spirit. The sample contained three times the quantity of spirit found in some beers.

The Solicitor for the defence contended that Section 3 of the Act provided for the exemption of medicated spirit, and if the Bench were satisfied that this was a medicated spirit and not a beverage, an excise certificate was not necessary. No druggist would be able to carry on his business if he were not allowed to sell this kind of article.

Mr. F. J. Lambert, for the prosecution, said that in some places druggists sold these wines, but where this was done without a licence an offence was being committed.

A fine of £15 with £8 19s. costs was imposed, but notice of appeal was given, and the magistrates agreed to state a case. Subsequently the intention to appeal was abandoned.

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### IDIOSYNCRASY TO A DRUG.

ON July 30 an action was brought at Dunfermline Sheriff Court against a Co-operative Society claiming £500 damages for illness alleged to have been caused by a headache powder sold by the defendants.

Medical evidence was given on behalf of the plaintiff to the effect that he was suffering from chronic gastritis due to an irritant powder.

The defence attributed the illness to the plaintiff's idiosyncrasy to aspirin, and called two medical witnesses to prove this.

The Sheriff found that the powder sold by the defendants consisted of 5 grains of phenacetin, 5 grains of aspirin, and 2 grains of caffeine citrate, that it was a pure drug and did not contain any irritant or other poison, and that it was not contaminated so as to be unfit for the purpose for which it was sold. The plaintiff had failed to prove any negligence at common law or any ground of liability under the Food and Drugs Act against the defendants.

Judgment was, therefore, given for the defendants with costs on the higher scale.

## The Quality of Paper.

### REPORT OF THE SPECIAL COMMITTEE SET UP BY THE LIBRARY ASSOCIATION.\*

THE Committee regard the institution of a recognised testing station and a recognised system of grade marks as fundamentally important. They emphasise the alarming extent to which books and documents are being written and printed on perishable papers, and they particularly condemn "Featherweight" and "art" papers. Four principal classes of paper are endorsed:—A, suited for permanence; B, for relative permanence; C, for general purposes where permanence is not essential; and D, for ephemeral productions. The first two grades are defined as:

*Grade I* for use where "absolute" permanence is required. This is divided into (1)*a*, all rag, hand-made tub-sized, and white, cream or unbleached linen or cotton rags of first quality (firsts); raw cotton or flax, or other pure cellulose only may be used for its manufacture, and the pulp is to be well washed. The least possible amount of alum consistent with the fairly hard sizing required is to be used, and the reaction is to be pH 5.5–6 *in situ*; no mineral loading matter is to be added, and iron salts should be present only in minute traces. Grade (1)*b*, as above, but the paper to be machine-made and tub-sized or engine-sized, or both.

*Grade II*, for long life but a less costly paper, is to be prepared from all-chemical-wood, to be machine-made and engine-sized. The pulp is to be well washed, the ash not over 5 per cent., alum to be the minimum necessary amount, rosin not over 1.5 or, at most, 2 per cent., mineral matter (added as loading) not above 5 or, at most, 6 per cent., and iron salts to be present only as traces. A paper intermediate to Grades I and II may be produced by admixture of rag with chemical-wood pulp. The ratio of prices would be Grade II = 1, Grade I (*b*) = 3, Grade I (*a*) = 6. A system of water marks for the various grades is suggested. The grades for archives are parallel to those for books.

D. G. H.

\* Obtainable from the Library Association, 20–27, Bedford Square, W.C.1. Price 1s. net.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Absence of Arachidic Acid from Cocoa Butter.** O. Lüning and W. Drude. (*Z. Unters. Lebensm.*, 1931, 61, 491–494.)—The experiments of Graf (*Arch. Pharm.*, 1888, 226, 843) indicate the presence of arachidic acid among the fatty acids of cocoa butter, but this has not been confirmed by subsequent work (Morgan and Bowen, *J. Soc. Chem. Ind.*, 1924, 43, 346*r.*; Amberger and Bauch, *ANALYST*, 1925, 50, 77). Graf's method was, therefore, applied to a number of cacao beans at least 30 years old (from Accra, Ariba, Carracas, Java, Porto Cabello, and Ceylon), and to extracted cocoa butter of known origin, large quantities

of fat being used and the fatty acids being fractionally precipitated as the magnesium salts (*loc. cit.*), the magnesium contents of which were then determined. When the magnesium compounds were dried at 95° C. until constant in weight, results were obtained indicating the presence of stearic acid but no higher fatty acids, and this was confirmed by repeated fractional precipitations and recrystallisations of the fatty acids from alcohol, and finally by melting point determinations of the fatty acids alone and in admixture with stearic acid. When, however, the magnesium salts were dried in a vacuum desiccator (Graf's method) the magnesium value was nearer that corresponding with arachidic acid. In one case (a suspected sample of milk chocolate) 0.97 grm. of arachidic acid was obtained from 20 grms. of fat, and this was attributed to adulteration with hardened arachis oil.

J. G.

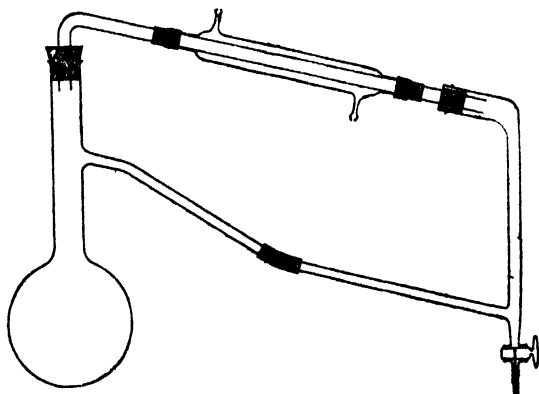
**Sapote (Mammy Apple) Seed and Oil.** G. S. Jamieson and R. S. McKinney. (*Oil and Fat. Ind.*, 1931, 8, 255-256.)—The sapote or "marmalade" tree of tropical America, *Calocarpum mammosum*, N.O. *Sapotaceae*, yields brown scaly fruits with pink pulp, 10-20 cm. long, containing one seed, weighing 20 to 26 grms., in which is a notable quantity of amygdalin. The seed examined contained 9.4 per cent. of moisture and 57 per cent. of a bright yellow oil which deposited stearine on standing, and the analysis of an 8-year-old sample of oil which had been stored at 2° C. gave the following results:—Sp. gr. at 25° C., 0.9105;  $n_D^{25}$ , 1.4652; saponification value, 189.2; iodine value (Hanus), 70.2; acetyl value, 12.2; Reichert-Meissl value, 0.15; Polenske value, 0.30; unsaponifiable matter, 1.39 per cent.; saturated acids, corrected, 30.37; and unsaturated acids, corrected, 63.73 per cent. The unsaturated acids were calculated to consist of 80.2 per cent. of oleic acid and 19.8 per cent. of linolic acid. The saturated acids were separated, esterified with absolute methyl alcohol in presence of dry hydrogen chloride, and the esters fractionally distilled under reduced pressure; the iodine value and saponification value of each fraction were determined, the free fatty acids recovered from portions of each of the fractions and their identity established. The acids consisted of palmitic, 30.95; stearic, 68.99; and arachidic, 0.06 per cent. Sapote oil is suitable for cooking, as a salad oil in tropical countries, or for soap.

D. G. H.

**Natural Phosphatide Content of Cacao Beans and its Change during Manufacture.** B. Rewald and H. Christlieb. (*Z. Unters. Lebensm.*, 1931, 61, 520-523.)—Comparative experiments were carried out to determine the effect on the phosphatide content of cacao beans of normal roasting and roasting for 2 hours at 120° C., with or without treatment with alkali, and of the pressure processes used for the removal of butter. The beans were finely ground, and 50 grms. were heated for 5 hours under a reflux condenser with a mixture of benzene and alcohol (80:20), the solvent being renewed 7 times until the sample was exhausted. The total extract was evaporated, the residue extracted with ether, and the resulting fat obtained after evaporation weighed and filtered, and the phosphatide content determined (details of method not given). The fat contents varied from 54.4 to

58.8 per cent., and the phosphatide, which was present in all the samples examined, from 0.057 to 0.238 per cent. Higher phosphatide contents in prepared products may be due to addition of other materials (*e.g.* nuts or milk to chocolate). None of the manufacturing processes mentioned produced any marked change in phosphatide content. Cocoa butter separated by pressure contains only a trace of phosphatide, since this is retained by the residual mass; the close association of phosphatide with protein matter observed with other foodstuffs is indicated (*cf.* Rewald, *ANALYST*, 1931, 113). J. G.

**Assay of Drugs yielding Essential Oils.** G. R. A. Short. (*Perf. and Ess. Oil Record*, 1931, 22, 208-210.)—The disadvantages of the various methods suggested for determining the volatile oil obtainable from spices by ordinary distillation are avoidable by the use of a 1-litre Pyrex distillation flask to which is attached a bent water-jacketed condenser tube having its end connected, by means of a cork with a small V-shaped incision to serve as a vent, with a receiving tube provided with a tap and with a side-tube. This side-tube is joined to that of the



flask by a short rubber tube, and thus acts as a flow-back for the aqueous portion of the distillate. For drugs giving less than 2 per cent. of oil, a larger flask should be used.

Sufficient of the drug (bruised or coarsely powdered if necessary) to yield 2 to 3 grms. of volatile oil, is placed in the flask with 500 c.c. (or 200 c.c. for oils giving 10 per cent. or more of oil) of water. The receiving tube is disconnected at the top and filled with water, after which 5 c.c. of petroleum spirit are added from a pipette. The flask is connected and distillation is carried on for 6 hours from a paraffin-wax bath. The whole contents of the receiving-tube are then drawn off into a small stoppered separating funnel, the tube being afterwards disconnected from the flask and condenser and rinsed out with two 5 c.c. portions of petroleum spirit. The rinsings are transferred to the separating funnel, which is left till separation is complete, the aqueous layer being then drawn off and the petroleum spirit extract filtered through a 90 ml. filter-paper (previously moistened

with petroleum spirit) into a tared 100 c.c. beaker flask. The filter is washed with 5 c.c. of the spirit and the beaker flask, containing the total filtrate, immersed in a water-bath at 50° C. to a level slightly above that of the liquid inside the flask. A steady current of air is passed over the surface of the liquid for 5 minutes, after which the flask is left in a desiccator over sulphuric acid and weighed to obtain the amount of oil extracted. By this procedure 100 grms. of cummin fruits yielded 2.39 to 2.40 per cent. of oil, the percentages found by distilling 3 and 5 kilos. in an ordinary steam-jacketed still being 2.40 and 2.43 respectively.

From four 20 gram. samples of cloves from the same bulk, 15.50, 15.80, 15.87, and 15.47 per cent. of oil were obtained, and from two samples from another lot, 10.85 and 11.00 per cent. By determining the quantity of volatile oil lost in the distillation (owing to its solubility in the water used) and that lost during the removal of the solvent, it is found that results the more nearly in accord with the amounts of oil actually present in the drug are obtained when no solvent is used. Such modification of the process, however, is possible only for oils which are readily separable, and is impracticable with cloves, pimento, parsley fruits, and myrrh.

T. H. P.

**Abnormal Aniseed Oils and B.P. Requirements.** W. M. Seaber and S. Marshall. (*Perf. and Ess. Oil Record*, 1931, 22, 163-165.)—A fair proportion of the star aniseed oils examined during the past year have shown abnormally low refractive indices (down to 1.5502 at 25° C.), in relation to their congealing and melting points. Comparison of normal (A) and abnormal (B) oils of almost identical melting points gave the following results:

	A.	B.
Specific gravity at 20° C. ..	0.9845	0.9820
Optical rotation .. ..	+0.25°	nil
Refractive index, $n_D^{25}$ C. ..	1.5530	1.5515
Congeeing point .. ..	15.9° C.	15.8° C.
Melting point .. ..	17.6° C.	17.4° C.

When distilled from the Wurtz flask with three bulbs recommended by the Essential Oil Sub-Committee of the Society of Public Analysts, the oils showed very similar boiling point ranges, but the first distillate (71 per cent.) and the residue (7.5 per cent., b.pt. above 245° C.) of B were of lower refractive index than the corresponding fractions of A, although the indices for the middle fraction (21.5 per cent., b.pt. 235° to 245° C.) were almost equal. No adulterant could be detected in B, and the anethole contents of the two oils appeared similar.

Examination of a larger sample of another abnormal oil, with refractive index 1.5515 (at 25° C.), congealing point 16.7° C., and melting point 18.4° C., revealed the presence of a fraction (7 per cent.) of low boiling point (210°-225° C.), and low refractive index (1.5283), which is absent from the normal oils, and is, in general, of terpene-like character. The results of (1) a rough estimate of the anethole-content by freezing and pressure, (2) distillation, and (3) examination of the ultra-violet absorption, indicate that the oil contains at least as much anethole as the usual commercial oil of similar melting point.

It appears that the lower limit of refractive index given in the British Pharmacopoeia of 1914 would result in the rejection of certain genuine oils, and it is suggested that this limit be lowered to 1.5510. T. H. P.

**Formula for the Determination of Saccharin by Titration.** O. Beyer. (*Chem. Ztg.*, 1931, 55, 509-510.)—On neutralisation (to phenolphthalein) of  $G$  grms. of a mixture containing only pure saccharin ( $x$  grms.) and para-saccharin ( $p$ -sulphamino-benzoic acid) with 0.1  $N$  potassium hydroxide solution ( $c$  c.c.), the compounds  $o$ - and  $p$ -  $C_6H_4.COOK.SO_3NH_2$  are formed, respectively. Since 54.59 and 46.69 c.c. are required, respectively, by 1 gram. of each of the pure saccharin compounds,  $x$  may be calculated from the equation  $54.59x + 46.69(G-x) = c$ , the para-saccharin ( $G-x$ ) being obtained by difference. In practice it is preferable to use 5 grms. of the sample and 0.5  $N$  alkali, and the error in the saccharin figure is then 0.1 to 0.3 per cent. J. G.

**Cocaine and Allylcocaine in Narcotics.** H. Emde. (*Chem. Ztg.*, 1931, 55, 537-539.)—For the examination of narcotic mixtures containing cocaine or its analogues, no general scheme is possible, as variations in such mixtures are continually being made. Mixtures of cocaine and novocaine may be investigated qualitatively by the taste, the solubility, the reaction of the solutions towards litmus, the behaviour towards certain alkaloid reagents, the diazo reaction, and reactions for anions (halogens, sulphate, nitrate). For quantitative examination, use is made of: the melting point; the optical rotation, before and after hydrolysis, and in aqueous and  $N$  hydrochloric acid solution; titration of a weighed amount of the aqueous solution with 0.1  $N$  sodium hydroxide solution in presence of phenolphthalein; isolation and characterisation of the precipitated bases, and determination of the anions in the filtrate. The exact procedure followed in certain cases encountered is described.

The taste of cocaine hydrochloride is somewhat bitter, and that of novocaine hydrochloride is saline. Both salts are dazzling white, but the former has a slightly blue, and the latter a faint yellowish tint. Those engaged in the contraband trade in these substances are said to be able to distinguish them, even in mixtures, by scattering a small quantity on a mirror and observing it from the side, the crystals of the cocaine salt showing faint iridescent colours, which do not appear with the novocaine compound; the author could not, however, confirm this difference.

Cocaine and other derivatives of natural *l*-ecgonine are laevo-rotatory in solution, whereas novocaine is optically inactive. Cocaine gives no specific reactions, although with the general alkaloid reagents it yields precipitates, these being similar to those formed by novocaine. By virtue of its arylamino group, novocaine gives a characteristic diazo reaction: when a solution of 0.1 gram. of the hydrochloride in 5 c.c. of water is treated with 2 drops of 25 per cent. hydrochloric acid and 2 drops of freshly-prepared 10 per cent. sodium nitrite solution, and the mixture is poured into a solution of 0.2 gram. of  $\beta$ -naphthol in 1 c.c. of 15 per cent. sodium hydroxide and 9 c.c. of water, a scarlet precipitate is obtained.

Examination of mixtures of cocaine and novocaine only by means of the chromic acid reaction for cocaine is followed in a certain country through which large quantities of these substances pass, but may lead to erroneous conclusions. A mixture is regarded as free from cocaine if its aqueous solution yields no crystalline precipitate of yellow cocaine chromate on addition of 3 per cent. aqueous chromic acid solution and a little hydrochloric acid. This precipitate is formed sometimes, but not always, with ordinary or methyl-cocaine, and also with allyl-cocaine. So long as natural ecgonine is used for the manufacture of cocaine and the rotation is not compensated by the introduction of dextro-rotatory substances, absence of rotation indicates absence of cocaine.

Information of value is obtainable by hydrolysis by means of *N* hydrochloric acid, cocaine then yielding ecgonine and benzoic acid, which crystallises out when the solution is cooled, even if the original cocaine is impure; this reaction is not disturbed by the presence of novocaine. Since 1 grm. of cocaine gives 0.4026 grm., and 1 grm. of allyl-cocaine 0.3708 grm. of benzoic acid, this reaction serves as a basis for quantitative determination, and may be used as a micro-test. Further details of the procedure are to be published elsewhere.

Methyl-cocaine (base) has m.pt. 98°C., and allyl-cocaine m.pt. 92°C. The allyl-base (and its salts) is less soluble than methyl-cocaine, and may be crystallised out from mixtures of the two. In 99 per cent. alcohol ( $c=2.0065$ ) it has  $[\alpha]_D^{20} - 31.6^\circ$ , and in *N* hydrochloric acid ( $c=4.999$ )  $[\alpha]_D^{20} - 47.8^\circ$ . Its hydrochloride, m.pt. 178°C. (swelling), is hygroscopic. A commercial Norwegian preparation showed  $[\alpha]_D^{20} - 46.5^\circ$ . In mixtures of methyl- and allyl-cocaines, as little as 0.1 per cent. of the methyl compound may be detected by oxidising the allyl derivative to benzoylecgonine by means of permanganate, 5 atoms of oxygen being required per molecule of allyl-cocaine. To the mixed bases, in sulphuric acid solution, 2 per cent. potassium permanganate solution is added, with constant stirring and at a temperature not exceeding 5°C., until a filtered portion of the liquid remains pink and clear for some minutes at 5°C. The solution is then filtered, made slightly ammoniacal, and shaken with ether or, better, petroleum spirit, until no further extraction takes place. The combined extracts are purified by shaking with a little water, and are afterwards shaken with successive small amounts of *N* hydrochloric acid, and finally with water, until all the bases are removed. The united hydrochloric acid and aqueous extracts are either completely or fractionally (according to the quantity of acid used) precipitated by 1 per cent. ammonia solution. The cocaine thus isolated is characterised by its m.pt. and optical rotation.

Commercial cocaine hydrochloride, conforming to pharmacopoeial requirements, always shows lower values (at most  $-70.5^\circ$ ) than the true value,  $-72^\circ$  for  $[\alpha]_D^{20}$ , in about 5 per cent. aqueous solution, owing to the presence of the ethyl-cocaine salt.

T. H. P.

**Chemical Characteristics of Colloidal Silver.** G. Gaume. (*J. Pharm. Chim.*, 1931, 123, 609-617.)—Colloidal silver may be considered of a good commercial quality if it conforms to the following specifications: The solid should be



of a grey-blue colour; it should rapidly yield a 0.1 per cent. slightly dichroic (red-green) aqueous solution, which gives no deposit after half-an-hour; the proportion of silver should be between 70 and 72 per cent.; a few c.c. of a concentrated (10 per cent.) solution evaporated at 50° C. should deposit brilliant, non-adhering plates; the alkalinity (as sodium hydroxide) should be between 1 and 1.5 per cent.; a mixture of 5 c.c. of a solution of 1 grm. of the silver in 999 c.c. of water with 5 c.c. of a solution of sodium chloride (1 grm. in 19 grms. of water), when shaken for 1 minute, should appear clear and red-brown in incident light and not blackish or opaque. Such a product is stable in light and under the action of air, and solutions may be kept for a long time without alteration.

D. G. H.

## Biochemical.

**Absorption of Aluminium Compounds.** Dee Tourtellotte and O. S. Rask. (*Amer. J. Hyg.*, 1931, 14, 225-230.)—A brief outline is given of the results obtained by other workers on the biological rôle of aluminium compounds. The work of McCollum, Rask and Becker (*J. Biol. Chem.*, 1928, 77, 753) and of Myers, Mull and Morrison (*J. Biol. Chem.*, 1928, 78, 595; *ANALYST*, 1928, 53, 547) showed that, (1) aluminium compounds are not toxic or detrimental to any biological function when present in diets in concentrations not exceeding 2 mgrms. of aluminium per rat per day, (2) aluminium compounds are not appreciably absorbed out of the intestinal tract from diets containing up to 600 parts per million of aluminium, and (3) animal tissues contain less than 2.0 parts per million of aluminium. However, Schaeffer *et al.* (*Bull. Soc. Sci. d'Hygiene aliment.*, 1928, 16, Nos. 1 and 2), and Underhill *et al.* (*Amer. J. Physiol.*, 1929, 90, 67) published results which were at variance with these. The authors have investigated certain of the disputed points. The investigation has involved (1) a critical study of the spectrographic method for the determination of aluminium in biological matter, including the development of certain modifications in the arc and spark techniques of spectral excitation, (2) the application of the modified spectrographic techniques to the determination of aluminium in biological and other materials, and (3) a re-investigation of the extent to which aluminium is absorbed out of the alimentary tract. The first two points (which are reported elsewhere) showed biological material to contain from less than 0.05 to about 1.0 parts per million of aluminium. This confirms the results of McCollum, Rask and Becker, and Myers *et al.*, but does not agree with the conclusions of Underhill *et al.* that biological materials may contain up to 100 p.p.m. of aluminium. The experimental data are now given for the investigation of the aluminium absorption and retention in rats given a diet which contained 0.6 per cent. of aluminium chloride, *i.e.* 600 parts per million of aluminium. The results show that the aluminium present in the diet is not absorbed and deposited as Underhill *et al.* contend, and that diets containing 600 p.p.m. of aluminium are not in any way injurious to growth and health, as suggested by Schaeffer *et al.* It may be said that the work of the authors confirms the earlier findings of McCollum, Rask and Becker, and of Myers *et al.*, and especially those which were disputed by Schaeffer *et al.* and by Underhill *et al.*

P. H. P.

**Effect of Ingested Cottonseed Oil on the Composition of Body Fat.** N. R. Ellis, C. S. Rothwell and W. O. Pool. (*J. Biol. Chem.*, 1931, 92, 385-398.)—Earlier work by Ellis and Isbell (*J. Biol. Chem.*, 1926, 69, 219; *ANALYST*, 1926, 51, 524) showed that the ingestion of cottonseed oil by hogs resulted in the formation of a firm body fat characterised by a high melting point and a high saturated acid content. A similar effect has been found in connection with the feeding of cottonseed meal. In order to determine the level at which maximum firmness was produced, experiments were conducted on hogs in which the oil was added to basal rations at 4, 8 and 12 per cent. levels. The hardest carcasses were secured on the 4 per cent. level. The higher quantities resulted in increasing softness. As the feeding experiments progressed, it became evident that an unusual transformation in the composition of the fat was taking place. The investigation was accordingly extended with the view of seeking the explanation for the metabolic process involved. Analysis of the lard showed striking changes in the fatty acid distribution. Among these was a marked increase in the linolic and the stearic acids at the expense of the oleic and palmitic acids. Although the maximum content of total saturated acids occurred in the 4 per cent. level, the maximum in stearic acid increased steadily up to the 12 per cent. level. It is suggested that the changes in composition of the lard are due to the presence in cottonseed oil of one or more isomeric forms of oleic or, possibly, linolic acid which are readily converted into stearic acid. Cottonseed oil feeding tests on rats gave a more pronounced softening of the body fat than was obtained in hogs. There were indications that changes in the fat composition occurred in the rat as in the hog following ingestion of cottonseed oil. One of the problems in the study of soft pork has been to find suitable hardening feeds available for use in conjunction with the softening feeds. Cottonseed meal is a valuable protein supplement for use with maize. The use of this feed was restricted for many years because of difficulties encountered when it was given to hogs. Lately, interest in this field has been revived, and experiments indicate that cottonseed meal may come into more general use as a hog feed. That it has hardening rather than softening properties adds to its value.

P. H. P.

**Progressive Changes in the Waxlike Coating on the Surface of the Apple during Growth and Storage.** K. S. Markley and C. E. Sando. (*J. Agric. Res.*, 1931, 42, 705-722.)—Discs from the sunny and shady side of a large number of representative samples of different varieties of apples at various stages of growth were immersed in dilute hydrochloric acid, and the thin layer of cuticle and its waxy covering with the adhering epidermal cells were removed, washed and dried, and the total ethereal extract, ursolic acid and oily fractions were determined in mgrms. per 100,000 sq. mm. of apple surface. In general, the shady side had larger quantities of ursolic acid and total ethereal extract in all stages of growth and during storage (indicating a more mature condition on the sunny side), and ursolic acid, total ethereal extract and oily fraction increased during growth and storage, the oily fraction at a faster rate than the ursolic acid.

The shift in the ratio of oily fraction to ursolic acid is undoubtedly accompanied by a change in the physical properties of the natural waxy coating, affecting the effectiveness of spray application and the ease of removal of the residue. Different varieties of apples show different figures on analysis, and variation may occur from year to year, probably depending on the favourableness or otherwise of the season to normal development. The average quantity of each substance deposited by the time the early stage is reached (40 to 60 days from blooming) considered as a percentage of the amount of the same substance present at maturity and at the end of the storage season, respectively, was: Ursolic acid, 75 and 68 per cent.; oily fraction, 50 and 45 per cent.; and total ethereal extract, 65 and 58 per cent.

D. G. H.

## Bacteriological.

**Chemical Changes in the Fat of Frozen and Chilled Meat. Part II. Chilled Beef.** C. H. Lea. (*J. Soc. Chem. Ind.*, 1931, 50, 215-220r (cf. ANALYST, 1931, 538).)—The carcase of a steer was placed while hot in a chamber at 0° C. of constant humidity  $78 \pm 2$  per cent., and after 2 days the sides were quartered. After 25 days, part of the carcase was removed, and kept at 10° C. for four days; part was left in the chamber for 42 days, and then kept at 10° C. for 4 days, and some of the fat was exposed to light of varying intensities. Visible colonies of moulds, yeasts and bacteria appeared on exposed muscle early in the experiment, but there was no general spreading of mould over the surface fat. *Pseudomonas* organisms were apparently responsible for a "tainted" odour first apparent on the 15th day, but later this gradually disappeared. Up to about 25 days the free acidity followed the normal curve for mutton fat at storage temperatures below 0° C., but later the acidity of the external fat increased rapidly, and at 42 days ranged from 5 to 11 per cent., although the kidney fat still contained less than 1 per cent. of free acids. Oxidation was very slow in absence of light. The flavour of the fat obtained, compared with that of control fat kept at -20° C., was good after 25 days, and fairly good after 42 days, but a definite taint had appeared in places. In a second experiment conditions were the same, but the air was agitated by fans whereby the growth of micro-organisms was much retarded, and the fat flavour was excellent after 42 days at 0° C., and there was only slight tainting after 60 days. In a third experiment storage was at a temperature slightly below the freezing point of the meat, without air circulation, and after 42 days the meat was allowed to hang at ordinary temperature in subdued light for 3 days. The only visible sign of micro-organisms was on the fore quarters, particularly at the neck; oxidation of external fat was negligible, and the flavour, except that of a small localised area of breast fat, was excellent. The "drip" was more than that which normally occurs with South American chilled beef, but it would be less for shorter periods of freezing. It is more difficult to chill successfully the fore quarter of beef than the hind quarter, and "taint" appears first in the fore quarter. Although a high free acidity is not of itself responsible for taint, whenever "taint" appears the free fatty acids are correspondingly high, and the "life" of chilled beef

appears to be determined by the growth of micro-organisms, and, in practice, a balance must be struck between excessive loss in weight of the carcase and deterioration of the fat.

D. G. H.

**Growth of Micro-organisms on Chilled and Frozen Meat.** R. B. Haines. (*J. Soc. Chem. Ind.*, 1931, 50, 223-227T.)—Counts taken of the number of micro-organisms on a carcase of lamb held at  $-5^{\circ}\text{C}$ ., show that on first freezing there is a big drop in numbers, followed by a steady fall of organisms having their optimum growth temperature at  $37^{\circ}\text{C}$ ., but on lean portions, after the initial drop, there is a marked rise in the figures obtained on gelatin media incubated at  $20^{\circ}\text{C}$ ., mainly due to yeast and mould growth. A temperature of  $-10^{\circ}\text{C}$  is considered satisfactory, as no rise then follows the initial drop. To store chilled meats successfully ( $0$  to  $-2.5^{\circ}\text{C}$ .), cleanliness of handling and control of humidity in the store are essential, and spoilage may occur through contamination with certain organisms such as one of the *Pseudomonas* group. Biochemical studies, in which the increase in "soluble nitrogen" (which is taken as a measure of the disintegrated products of the muscle proteins), is followed, show that the disintegration of samples incubated at  $37^{\circ}\text{C}$  varies greatly, but that a temperature of  $0^{\circ}\text{C}$  is clearly not enough in itself to inhibit bacterial action. Immediate cooling of carcases intended for holding for long periods in the chilled state, or in cold store, is advocated, so that the growth of bacteria may be checked, and also the production of soluble nitrogenous matter (due to autolysis), which would serve as food supply for the initial or any subsequent inoculation.

D. G. H.

## Toxicological.

**Distribution of Gallium in the Organism.** C. Levaditi, J. Bardet, A. Tchakirian, and A. Vaisman. (*Compt. rend.*, 1931, 193, 117-119.)—In view of the therapeutic action of gallium in cases of trypanosomiasis and of experimental syphilis of the rabbit, the distribution of this metal in the bodies of rabbits to which the tartrate was administered in doses varying from  $0.015$  to  $0.06$  grm. per  $1000$ , has been investigated. The blood and tissues, dried at  $110^{\circ}\text{C}$ ., were incinerated, and the ash, after being washed with water and ignited, was examined (1) by measuring the absolute intensities of rays of the arc-spectrograms, and (2) by determining the time taken for the bluish-violet coloration with resorcinol and ammonia to appear.

The results show that gallium persisted in the organism for at least 69 days after injection of the tartrate. The liver and blood always contained appreciable quantities of the metal, and the highest proportions were found in the kidneys. Of four brains examined, only one showed traces of gallium. The presence of gallium in the urine of a man under treatment is in accord with its concentration in the renal filter. Thus, the distribution of gallium in the organism is similar to that of bismuth, tellurium, or mercury.

T. H. P.

## Organic Analysis.

**Studies on the Starch-Iodine Reaction.** J. Field. (*J. Biol. Chem.*, 1931, 92, 413-419.)—The usefulness of the radiomicrometer in the study of reactions involving a marked colour change was indicated previously by Field and Becking (*J. Gen. Physiol.*, 1925-26, 9, 445). It was then shown that the starch-iodine reaction could be investigated in this way. Further observations on the reaction, bearing on the problem of the chemical specificity of some starches and upon the nature of starch-iodide, are now reported. It has been shown by radiomicrometric titration that clear solutions of the cold water-soluble fractions of ground maize and wheat starches, have, at a given concentration, approximately the same capacity for adsorbing iodine. This fact indicates a lack of specificity of these preparations in so far as this property is concerned. The significance of dilution as a factor in the determination of the iodine-binding capacity of these preparations supports the conclusion that starch-iodide is an adsorption compound.

P. H. P.

**Use of Arylcarbimides in Identifying Hydroxylic Compounds.** G. T. Morgan and A. E. J. Pettet. (*J. Chem. Soc.*, 1931, 1124-1126.)—Phenylcarbimide is prepared (Hentschel, *Ber.*, 1884, 17, 1284) by passing carbonyl chloride over fused aniline hydrochloride in a lagged retort, followed by decomposition by heat of the resulting solid phenyl carbamyl chloride, and is purified by repeated distillation. Like *α*-naphthylcarbimide (French and Wirtel, *J. Amer. Chem. Soc.*, 1926, 48, 1736), it has been used to separate and characterise phenols in tar products (Morgan and Pettet, *J. Soc. Chem. Ind.*, 1931, 50, 72), but both reagents have the disadvantage that many of the derivatives are oily products. *p*-Xenylcarbimide yields less fusible derivatives ( $C_6H_5.C_6H_4.NH.COOR$ ) from which the original hydroxyl compound may be regenerated by boiling with dilute aqueous or aqueous alcoholic ammonia. It is prepared by passing carbonyl chloride into a solution of 60 grms. of *p*-xenylamine (see Morgan and Walls, *id.*, 1930, 49, 15) in 1500 c.c. of warm toluene, which is then maintained at the b.pt. for 2 hours until no more hydrogen chloride is evolved. The solution is filtered, toluene removed by distillation, and the residual reagent recrystallised from petroleum spirit (b.pt. 40 to 60° C.); it has m.pt. 56° C., and b.pt. 283° C. (decomp.). The reaction is carried out by heating the hydroxy-compound at 100° C. for 1 hour with a slight excess of reagent, the cold, solid mass being crystallised from alcohol and so separated from *s*-di-*p*-xenylcarbamide. The m.pt. (°C.) of the phenylcarbimates and *p*-xenylcarbimates are:—Methyl alcohol, 47, 127 (the latter is precipitated in gelatinous form which subsequently becomes crystalline); ethyl alcohol, 51, 119 (the latter behaves similarly to the methyl alcohol derivative); *n*-propyl alcohol, 57, 129; *iso*-propyl alcohol, 90, 138; *n*-butyl alcohol, 55, 109; *n*-amyl alcohol, —, 99; cyclohexanol, 83, 166; phenol, 126, 173; *o*-cresol, 144, 151; *m*-cresol, 125, 164; *p*-cresol, 115, 198; benzyl alcohol, 78, 156; *o*-4.xylenol, 120, 183; *m*-4.xylenol, 112, 184; *p*-xylenol, 162, 162; thymol, 106, 194; carvacrol, 135, 166; *α*-naphthol, 178; 190; catechol, 169, decomp.; resorcinol, 164, decomp.; quinol, 224, decomp.; homocatechol, 166, 193; orcinol, 154, 196.

J. G.

**Titration of Hydroxy Organic Acids in the Presence of Ferric and Cupric Salts.** C. V. Smythe. (*J. Biol. Chem.*, 1931, **92**, 233-244.)—In previous work by Smythe and Schmidt (*J. Biol. Chem.*, 1930, **88**, 241) and Smythe (*J. Biol. Chem.*, 1931, **90**, 251) it was pointed out from theoretical reasoning that when a hydroxy organic acid combines with a metal, such as iron, to form a non-ionised compound the acid properties of the alcoholic hydroxyl group must be increased. There is abundant evidence in the literature which indicates that the hydrogen of such hydroxyl groups may be replaced by a metal, but there is apparently no direct evidence showing how strongly acidic these groups become. Titration curves, obtained by means of the glass electrode, are now reported for ferric chloride, for cupric chloride, for each of the following acids, lactic, glycollic, oxalic, malic, tartaric and citric, and for each of these acids in the presence of ferric chloride and in the presence of cupric chloride. It is shown that the presence of these metal salts greatly alters the titration curves; the mixtures of acid and metal chloride are considerably more acidic than either of these substances alone. The nature of this change is discussed in detail, and the application of the results is pointed out. Two explanations, based on the interaction of colloids and non-colloids, respectively, are advanced. When ferric chloride is titrated the iron is precipitated as ferric hydroxide, but no precipitate is formed when the mixture (acid and ferric chloride) is titrated. Possibly the ferric hydroxide is formed and stays in colloidal solution. The other explanation is that when the acid and iron combine to form a complex, the alcoholic hydroxyl group takes part in the combination; this action makes the oxygen of the group more positive, and, as a result, the hydrogen must become more acidic. This general explanation applies to all the six acids used.

P. H. P.

**Sensitive Colour Reaction for Certain Quinones.** R. Craven. (*J. Chem. Soc.*, 1931, 1605-1606.)—More than 0.01 mgrm. per c.c. of *p*-benzoquinone or its dichloride, *o*-toluquinone, chloranil,  $\alpha$ -naphthoquinone or thymoquinone gives an intense bluish-violet colour, changing (rapidly with *p*-benzoquinone) through blue and green to red-brown, on addition of 3 drops of ethyl cyanoacetate and 3 c.c. in excess of a mixture of equal parts of absolute alcohol and ammonia (sp. gr. 0.880). Since  $\beta$ -naphthoquinone, anthraquinone, 1:5-dichloro-2:6 diamino-benzoquinone (chloranilamide) and phenanthraquinone do not react, the presence of a labile hydrogen or halogen atom adjacent to the carbonyl group of the quinone is indicated as essential for the reaction. Some of the condensation products have been isolated.

J. G.

**Oil from Linaloe Husks from India.** Anon. (*Bull. Imp. Inst.*, 1931, **29**, 182-183.)—The fully ripe fruits of trees probably of the species *Bursera Delpechiana*, grown in Bangalore from Mexican seed, produced about 16.7 per cent. of dried husk which, on distillation, yielded 18.1 per cent. of oil giving the following constants:—Sp. gr. (15.5° C.), 0.8989;  $[\alpha]_D^{20}$ ,  $-4.68^\circ$ ;  $n_D^{20}$ , 1.455; acid value, 0.7; ester value, 216.7; soluble in 3.3 volumes of 70 per cent. alcohol at 15° C. These values, particularly the high sp. gr. and low refractive index, indicate the predominance

of linalyl acetate in the oil, as distinct from Mexican commercial linaloe oil from the same species, which consists principally of free linalol. The difference in the sign of  $[\alpha]$  observed by previous workers for the fruit and wood oils of the linaloe tree (which are otherwise identical) is not confirmed. The oil is similar in composition to, and has the same commercial value as "Cayenne linaloe oil," which, however, is obtained from the wood of a tree of different botanical species (probably *Ocotea caudata*, Mez). J. G.

**Oil of Sumac.** H. P. Trevethick. (*Oil and Fat Ind.*, 1931, 8, 221.)—Sumac berries, used as a source of tannin, yielded on extraction with light petroleum spirit 17.54 per cent. of a greenish-brown, rather viscous oil, with the following characteristics: Moisture and volatile matter at 105° C., 0.18 per cent.; sp. gr. at 15.5° C., 0.9256; saponification value, 183.2;  $n_D^{20}$ , 1.4726;  $n_D^{20}$ , 1.4744; iodine value (Wijs), 96.1; free fatty acids, 10.7 per cent.; unsaponifiable matter, 2.38 per cent.; total fatty acids and unsaponifiable matter, 95.1 per cent.; iodine value of fatty acids, 96.7; neutralisation value, 193.2; titre, 25.7° C. D. G. H.

**Determination of Amines in Tannery Lime Liquors.** E. K. Moore, J. H. Highberger, R. Koppenhoefer, and F. O'Flaherty. (*J. Amer. Leather Chem. Assoc.*, 1931, 26, 341.)—Volatile alkalis in lime liquors, and also in commercial monomethylamine, consist of ammonia, mono-, di- and tri-methylamine. The ammonia is separated from the total volatile bases by shaking the whole with yellow mercuric oxide. The separation of the remaining bases depends on the different behaviour of each with nitrous acid. Methylamine forms methyl alcohol and nitrogen, whilst dimethylamine forms nitroso-dimethylamine, from which dimethylamine may be regenerated by reduction with zinc and hydrochloric acid. Trimethylamine forms trimethylamine nitrite, from which trimethylamine may be recovered by distillation with excess of sodium hydroxide.

A volume of the settled lime liquor containing volatile bases equivalent to 60 to 70 c.c. of 0.1 *N* acid is distilled into 50 c.c. of 0.2 *N* sulphuric acid at atmospheric pressure until two-thirds of the total volume have passed over. The excess acid is titrated with 0.1 *N* sodium hydroxide solution (methyl red as indicator), and from this value, the volatile base is calculated as moles per litre of lime liquor. The neutralised distillate is diluted to 250 c.c. (Solution A). One hundred c.c. of solution A, diluted to 230 c.c., is treated with 5 c.c. of concentrated sodium hydroxide and carbonate solution, diluted to 250 c.c., and poured into a brown glass-stoppered bottle containing 3 grms. of yellow mercuric oxide. The bottle is wrapped in a black cloth and shaken for one hour, after which the stopper is replaced by one holding a tube of cotton wool and an air inlet-tube, thus enabling the solution to be filtered by simple inversion into a receiver. Two hundred c.c. of this filtrate (80 c.c. of solution A) are distilled into standard acid and back-titrated, the difference between this value and the value before treatment representing the ammonia. For the determination of the trimethylamine, 100 c.c. of solution A are placed in a 500 c.c. Kjeldahl flask and made acid, evaporated to

20–25 c.c., and cooled. Twenty c.c. of saturated sodium nitrite solution and 10 c.c. of glacial acetic acid are added, mixed, and the whole allowed to stand 45 minutes, after which the bulk of the oxides of nitrogen is removed from the space over the solution by blowing, and a few drops of phenolphthalein solution are added. Thirty-two c.c. of a 20 per cent. sodium hydroxide solution are added so as to wet the neck of the flask evenly. (After carefully mixing the solution it should still be acid.) The flask is stoppered and left for 45 minutes, during which time the oxides of nitrogen will be absorbed. An excess of sodium hydroxide is added, and the liquid distilled into standard acid until only 25 c.c. remain undistilled. The back-titration is a measure of the trimethylamine.

The dimethylamine and trimethylamine are determined as follows:—The titrated distillate thus obtained is treated with 20 grms. of zinc and 10 c.c. of concentrated hydrochloric acid, then made alkaline with sodium hydroxide and distilled into standard acid. The proportion of total volatile bases due to ammonia, di- and tri-methylamine being known, that due to monomethylamine is found by difference. The ammonia determination is subject to error and is being further investigated. The error in the determination of amine does not exceed 3 to 5 per cent. and the method is quicker than that of Weber and Wilson, and can be carried out without the use of expensive apparatus. R. F. I.

## Inorganic Analysis.

**Specific Test for Zirconium.** F. Feigl, P. Krumholz and E. Rajmann. (*Mikrochem.*, 1931, 9, 395–400.)—Zirconium salts react with arsenic acid and its organic derivatives. Of a large number of organic arsenic compounds tested, *p*-dimethyl-aminoazophenylarsenic acid has been found to be the most suitable as a reagent for zirconium. *Preparation of the reagent.*—Twenty-two grms. of arsanilic acid are dissolved in 200 c.c. of 1:10 hydrochloric acid, diazotised with the calculated quantity of sodium nitrite, and mixed with a solution of 12 grms. of dimethylaniline in 2 *N* hydrochloric acid. After cooling with ice for an hour sodium acetate is added, and the precipitate is recrystallised from hot 2 *N* hydrochloric acid. The yield is 20–25 grms. of the hydrochloride. *Test.*—Thick pure filter paper is impregnated with a solution made from 0.1 gm. of the reagent in 5 c.c. of concentrated hydrochloric acid and 100 c.c. of alcohol, and is then dried. On placing a drop of a zirconium salt in acid solution on the filter paper, large amounts of the metal give a brown fleck at once. When less zirconium is present the reagent is quickly removed by placing the paper in 2 *N* hydrochloric acid at 50–60° C., when the brown fleck or ring of the zirconium becomes clearly visible. The smallest amount detectable is 0.1 $\gamma$  of zirconium, and the limit of dilution is 1:500,000 in 1 *N* hydrochloric acid. Sulphuric acid reduces the sensitiveness of the test, and phosphates, fluorides and organic salts which give stable complex salts with zirconium hinder the reaction. The alkalis and alkaline earths and aluminium, zinc, cobalt, nickel, chromium, manganese, mercury, bismuth, cadmium, copper, lead, thallium, silver, platinum<sup>(IV)</sup>, palladium<sup>(II)</sup>, cerium<sup>(III)</sup>



uranium, lanthanum, praseodymium, neodymium, and vanadium, do not react with the reagent when in 0.5–1.0 per cent. hydrochloric or nitric acid solution. Iron and antimony<sup>(III)</sup> salts give a brown colour, which is removed at once by soaking in hydrochloric acid, but in the presence of antimony<sup>(V)</sup> 2 or 3 minutes' soaking is necessary, when 0.2% of zirconium can be detected in the presence of 1000 times its weight of antimony. Thorium and gold salts in concentrated solution give a colour disappearing at once in hydrochloric acid. Molybdates, tungstates, tin<sup>(IV)</sup>, and titanium salts give a brown to red precipitate which is stable to acids. When these substances are present the test solution is mixed with concentrated hydrochloric acid and 30 per cent. hydrogen peroxide [when tin<sup>(IV)</sup> only is present the hydrogen peroxide is omitted] before placing on the impregnated filter paper, which is then placed in a bath of warm acid. The zirconium is then precipitated as a brown ring round a central zone. As much as 50 times the amount of titanium, 1000 times the amount of molybdenum, 500 times the amount of tungsten, and 250 times the amount of tin, may be present. Tantalum is the only metal which interferes with the test, giving an analogous reaction. J. W. B.

**Separation and Determination of Osmium. R. Gilchrist.** (*Bureau of Standards J. of Research*, 1931, 6, 421–448.)—The methods for the determination of osmium are reviewed. Ogburn and Miller's strychnine process (*ANALYST*, 1930, 55, 222) was tried and found to be unreliable. It was found possible to detect traces of osmium in presence of nitric acid by Tschugaeff's thiourea test: The solution is distilled with nitric acid in a current of air, and the distillate received in 6 *N* hydrochloric acid saturated with sulphur dioxide and containing a little thiourea. With more than 1 part osmium in 2,500,000 of solution, a pink colour develops in less than 15 minutes, the liquid being heated on a steam bath.

The following procedure is recommended for the separation from the other platinum metals: the solution (100 c.c.) is placed in a distilling flask (700 c.c.), the ground-glass stopper of which carries a tube through which air may be bubbled through the solution, a thistle funnel with glass tap, and an exit tube leading into the ground-glass stopper of the first condensing flask (300 c.c.). Two other similar condensing flasks are connected in series with the first. The four glass stoppers with their inlet and outlet tubes form one rigid piece. The three condensing flasks are charged with a saturated solution of sulphur dioxide in 6 *N* hydrochloric acid (150, 50, and 50 c.c.). Nitric acid (40 c.c., 1 : 1) is added through the thistle funnel, a slow air current made to pass through the train, and the solution distilled for one hour. This time suffices for the complete volatilisation of osmium from osmate and bromo-osmate solutions, but chloro-osmate solutions must be distilled for 7 to 8 hours. The combined liquors from the condensing system are evaporated as far as possible in a beaker on the water-bath, and the residue digested for 15 minutes with 10 c.c. of strong hydrochloric acid, which in turn is evaporated. Digestion and evaporation with hydrochloric acid are repeated three times more, so that any sulphite compound may be entirely decomposed. The last residue is taken up with 150 c.c. of water, which is heated to

boiling, and a solution of sodium bicarbonate added until a precipitate forms which suddenly coagulates. After addition of a few drops of bromphenol blue, enough bicarbonate solution is added to produce a faint bluish colour, and the solution boiled 5 to 6 minutes. The hydrolysis precipitate, which consists of hydrated osmium dioxide, is left to settle, and the liquid decanted through a tared Munroe crucible. The precipitate is transferred to the crucible and washed with a saturated solution of ammonium chloride. The platinum cap is fitted to the bottom of the crucible, which is covered with a perforated silica lid. The ignition is started very gradually in a stream of hydrogen. After 10 minutes' strong heating the crucible is cooled in hydrogen, which is displaced by carbon dioxide. The metallic osmium is weighed.

The following additional data on the conversion of osmium compounds into the tetroxide were obtained by experiment: Chloro-osmate solutions evolve all the osmium as tetroxide when heated with sulphuric acid near its boiling-point; bromosmate solutions distilled with sulphuric acid give off very little tetroxide. Either compound is readily converted into tetroxide when heated with a mixture of sulphuric and nitric acids. Ruthenium is not volatilised by nitric acid up to 40 per cent. by volume, nor by strong sulphuric acid; a mixture of nitric and sulphuric acids causes a certain amount of volatilisation. W. R. S.

**Colorimetric Determination of Traces of Ammonia. K. G. Makris.** (*Z. anal. Chem.*, 1931, **84**, 241-242.)—The reaction with tannin and silver nitrate (Makris, *ANALYST*, 1930, **55**, 715) is applicable to the colorimetric determination of ammonia as follows:—The water to be tested (250 to 300 c.c.) is distilled with magnesium oxide, and the first 50 c.c. collected. The standard solution is prepared by dilution of 1 c.c. of 10 per cent. ammonia to one litre; 100 c.c. of that solution are in turn made up to 1000. Ten Nessler tubes are successively filled with 1 to 10 c.c. of standard solution, and the volumes adjusted to 10 c.c. with distilled water. Ten other Nessler tubes are filled each with one c.c. of distillate and 9 of water. One standard and one assay tube at a time are treated at the same moment with 2 drops of pure 5 per cent. tannin solution and one drop of 20 per cent. silver nitrate solution, and the pale yellow to orange tints compared after one minute (for quantities of 0.005 to 0.04 mgrm. of ammonia, 3 to 4 minutes should be allowed). The comparison is then carried out with the other pairs of tubes until two tubes are found which yield equal tints. If the depth of colour of the unknown solution is intermediate between those of two consecutive standards, five fresh standard tubes are prepared in which the amount of solution increases at the rate of 0.2 c.c., and the matching is repeated. If one c.c. of distillate contains more than 0.0001 gm. of ammonia, the distillate should be diluted. The matching should be done at the times indicated, as the colour darkens after some time. W. R. S.

**Gravimetric Determination of Acetylene from Calcium Carbide. A. A. Wassiljew.** (*Z. anal. Chem.*, 1931, **84**, 217-220.)—The following gravimetric method, unlike the gasometric, does not require special apparatus, and utilises a

large quantity of sample, which is desirable on account of the difficulties encountered in obtaining an average sample of calcium carbide. A round-bottomed flask (750 c.c.) is closed with a well-fitting doubly-perforated cork stopper carrying a 150 c.c. separating funnel and an exit tube bent at 90°. This tube is connected with a drying tower, and this, in turn, with a large U-tube, both filled with granulated calcium chloride. The funnel is filled with 100 c.c. of 20 per cent. sodium chloride solution, and the weighed sample (60 to 80 grms.) is placed in the flask, which is corked and the corks painted over. The whole is weighed, placed in a draught cupboard, and the salt solution admitted dropwise, through the funnel, very slowly at first, later at the rate of 6 to 8 drops per minute; at the end it can be added in portions of one c.c. The flask is shaken from time to time. The reaction takes 3 to 5 hours, but requires little supervision. The amount of salt solution weighed in at the start is not sufficient to decompose the carbide, a small funnel being purposely used to keep the weight of the apparatus down. During the test, further quantities of salt solution are added as required from a flask weighed before and after use, the weight being added to that of the apparatus. When the contents of the flask form a thinnish homogeneous liquid, the apparatus is again weighed, the difference being  $C_2H_2$ . The solubility of the gas in the salt solution is very low. The method is suitable for laboratories where only an occasional determination of this kind is required; the results compare well with those obtained by the gasometric method.

W. R. S.

## Microchemical.

**Qualitative Micro-Electrolysis with a Small Electrode.** H. J. Brenneis. (*Mikrochem.*, 1931, 9, 385-395.)—Three forms of micro-electrodes—a "stick form" electrode, a "needle form" electrode, and an electrolytic microscope slide—are used for the detection of amounts of copper, silver, gold and lead of the order of 0.001-0.005 $\gamma$  of the element. For the "stick form" electrode fine platinum or platinum iridium wire is used. One pole is placed inside a fine glass capillary and the other outside, so that, except at the tips, they are separated by glass. Both are enclosed in a second thick-walled glass capillary, of 5 mm. outside and 0.5 mm. inside diameter. This serves to protect the electrodes and makes the apparatus easy to handle. The "needle" form electrode is similarly made, except that the outer glass capillary is thin-walled, and is thus more suitable for inserting into micro-apparatus. The electrolytic microscope slide (made by K. Schmitt, *Mechaniker*, Tech. Hochschule, Graz) is a similar electrode made on a microscope slide, but is 3 polar, and is therefore useful for simultaneous anode and cathode separation, such as the separation of copper from lead. The third pole has no current passing through, but is used for comparison. This type is particularly useful for watching the electrolysis under the microscope. Small drops of solution of 1 c.mm. to 0.1 c.mm. are taken for the tests, in which a normal circuit giving a suitable voltage (1.35 to 3 volts) is used. The time of electrolysis depends on the amount of substance present, and varies from a few seconds to

24 hours for a blank test, when a damp chamber must be used to prevent evaporation. When the metal separated on the anode or cathode cannot be identified with certainty under the microscope a confirmatory test must be made; gold can be seen to be insoluble in concentrated nitric acid, silver forms silver sulphide on being held over an ammonium sulphide bottle, and other micro-tests may be used. The electrodes have also been used for the detection of mercury, cadmium and zinc. A table is given of the tests for copper, silver, gold and lead, showing the voltages used, the composition of the electrolytic solutions, time of electrolysis, smallest amount detectable, and other details.

J. W. B.

**Quantitative Micro-analytical Determination of certain Hydroxy-acids by means of the Photo-electric Cell.** A. S. Williams, R. H. Muller and J. B. Niederl. (*Mikrochem.*, 1931, 9, 269-294.)—The colour produced in the reaction between certain hydroxy acids and ferric chloride is used in a method of determining the acids, and this method has been applied to *lactic acid*, as an example of a mono-hydroxy mono-basic acid, to *citric acid* as a mono-hydroxy polybasic acid, to *tartaric acid* as a poly-hydroxy polybasic acid, and to *salicylic acid* as an aromatic mono-hydroxy mono-basic acid. As the colour developed is sometimes very faint, rendering visual methods impracticable, the intensity is measured, using a photo-electric cell of the Müller and Partridge type (*Ind. Eng. Chem.*, 1928, 20, 423). It is important that the light source, a 6-volt Mazda lamp, should remain constant. It was found necessary to check the constancy in experiments over a period exceeding an hour by testing against a series of 7 standard alkaline solutions of potassium chromate, of 0.05 molar concentration, and diluted with 1 to 40 parts of distilled water. The strength of illumination is adjusted, if necessary, by means of a rheostat in the circuit.

**Procedure.**—For each measurement 10 c.c. of solution is used in a test tube which is always placed in the colorimeter in the same relative position, to obviate errors due to inequality in wall thickness. The maximum and minimum range of the light source are determined by taking readings with the cell screened from light and fully illuminated. The readings of the standard chromate solutions are taken, followed by the approximate reading for the test solution. The standard giving the reading nearest to the test solution is then re-measured; then, if necessary, the illumination is adjusted, and finally the test solution is measured accurately. The ferric chloride solution used for each test is also measured. The ferric chloride solution is made up from 2.7022 grms. of the hexa-hydrate in a litre of 0.005 *M*-hydrochloric acid. When the concentration of hydrochloric acid is lower the solution hydrolyses, and consequently cannot be used for reliable readings. For each acid a number of readings is taken on solutions of known concentration and from these a curve is constructed. The values for unknown solutions are read off from the curves. The accuracy of the determinations was found to be as follows:—Lactic acid, on 0.1–0.7 mgrm.  $\pm 1.3$  per cent.; citric acid, on 0.2–1.6 mgrm.,  $\pm 0.4$  per cent.; tartaric acid, on 0.3–1.8 mgrm.,  $\pm 1.0$  per cent.; salicylic acid, on 0.2–0.7 mgrm.,  $\pm 0.7$  per cent.; sodium salicylate, on 0.4–0.8

mgm.,  $\pm 2.0$  per cent. Calibration curves are given, and diagrams of the apparatus and electrical circuit.

J. W. B.

**Contributions to the Micro-Determination of Cholesterol.** M. Yasuda. (*J. Biol. Chem.*, 1931, 92, 303-312.)—Recently Okey (*J. Biol. Chem.*, 1930, 88, 367; *ANALYST*, 1930, 55, 654) described a micro method for the determination of cholesterol, in which the oxidative method of lipid determination of Bloor (*J. Biol. Chem.*, 1928, 77, 53) was applied to the cholesterol digitonide. The need of a micro method for cholesterol determination other than the colorimetric one has been recognised for a long time, and Okey's test seemed to fill the need. However, after repeated trials, it was found that when tissue extracts were used the results were not so good as when pure cholesterol solutions were used. In practice, the method had at least two disadvantages, which have been largely, if not completely, removed, by the modifications described. The oxidation of cholesterol or cholesterol digitonide, however, is not as easy as is the determination of phospholipid and fatty acid, and the conditions of oxidation of cholesterol have, therefore, been modified. The modifications of Okey's procedure may be summarised as follows: (a) By the use of acetone the separation of cholesterol digitonide from the excess of digitonin and lipids is greatly facilitated. (b) By the solution of the digitonide in hot absolute alcohol and filtration it can be entirely freed from impurities and delivered in a convenient form for further treatment. The time of oxidation of cholesterol digitonide was changed from 20 to 60 minutes at temperatures of  $124 \pm 2^\circ \text{C}$ . The whole procedure is considerably simplified, and the modified method can be carried out easily and quickly and gives accurate results. Comparative results by the colorimetric and modified micro-oxidative methods on tissue extracts show that the colorimetric method gives values about 15 per cent. higher than the oxidative method, and that the difference is not the same for all tissues. In liver the differences are in most cases between 15 and 20 per cent., in spleen about 15 per cent., in skin about 20 per cent., and in kidney, brain, tumour, and muscle under 10 per cent.

P. H. P.

**Erratum.**—The name of one of the authors of the paper in the August issue (p. 508) should be "S. Marks" instead of "L. Marks."

## Reviews.

**A LABORATORY MANUAL OF ELECTRO-CHEMISTRY.** By ERICH MÜLLER. Translated from the fourth German edition by H. J. T. ELLINGHAM. Pp. 363. Routledge & Sons, Ltd. 1931. Price 15s.

The book under review aims at providing a systematic course of practical instruction covering almost the whole field of electro-chemistry; the experiments described are those in use at the Technische Hochschule at Dresden, which has become famous as a school for the training of electro-chemists. The original German issue of this book passed through four editions between 1912 and 1924, and, consequently, it must have satisfied a definite demand on the Continent; there has, however, hitherto been no manual of practical electro-chemistry available in the English language.

Professor Müller's book contains full descriptions, including short theoretical discussions, of some seventy experiments; these are divided into the following six sections: Some Fundamental Laws, Deposition of Metals, Inorganic Preparations, Organic Preparations, Electrolysis of Fused Salts, and Electrothermal Processes. It is evident from these titles that very considerable ground is covered in this book. The conditions in British Universities do not permit of the completion of such a comprehensive course, but a choice of typical experiments might well be made with great advantage.

It seems somewhat regrettable to the reviewer that the 1924 German edition has been translated without any attempt to bring it up to date, except for the inclusion of an appendix dealing with modern developments of the theory of electrolytic dissociation. The translator has done his work well, but he would have performed a greater service to physical chemistry if he had included in the book descriptions of modern forms of apparatus. Dr. Ellingham is perfectly competent to make these additions, and so it is unfortunate that he has missed this opportunity. At present the book contains no mention of valve oscillators, of the various new types of hydrogen electrode, of the quinhydrone electrode, of the use of galvanometers in place of the temperamental capillary electrometer, or of modern potentiometers. In spite of these omissions the book does fill a gap in our scientific literature, and every chemist will find it a useful addition to his library; it is well printed on good paper, and has a sufficient number of clear diagrams.

S. GLASSTONE.

**HANDBUCH DER PFLANZENANALYSE.** Edited by VON G. KLEIN. Vol. I, General Methods of Plant Analysis. Pp. xii + 627. Vienna: Julius Springer, 1931. Price, unbound, R.M. 66.

This is the first volume of a work which promises to be the most comprehensive of any yet published on the subject with which it deals. The editor is a biochemist

of high reputation, and he has been fortunate in securing the services of competent writers for the different chapters into which the text is divided. The contributors, in addition to the editor, are R. Brieger, F. Feigl, P. Hirsch, E. Keyssner, H. Kleinmann, G. Kögel, H. Leib, H. Linser, J. Matula, L. Michaelis and C. Weygand.

The text contains 323 illustrations, and these render the minutest details of the methods described as clear and intelligible as is possible in words. The highest praise must, indeed, be accorded to the treatment of the subject matter throughout the volume. Having said so much in praise of Professor Klein's *Handbuch*, we now venture to offer some criticism.

Admirable though the volume is, much of the text deals with matter that could hardly be expected to be found in a work dealing with a specific subject. Our confrères in mid-Europe when they produce a manual, such as the one before us, aim at making it encyclopædic. This practice increases our already all too voluminous chemical literature; it obscures the matter properly relevant to a specific treatise; and it involves waste of time and money. The present volume errs in this direction. In justification of these remarks a few instances may be cited.

Part I, covering 515 pages, deals with such general matters as the qualitative testing of reagents for purity, the balance, ignition and boiling, evaporation, crystallisation, micro-methods for elementary analysis, and determination of molecular weight. These are only a few instances of subjects ancillary to the analysis of plants, and references to works dealing with them would have sufficed. On the other hand, centrifuging, ultra-filtration, dialysis and electro-dialysis are, so frequently employed in biochemistry that they cannot be regarded as extraneous to the subject matter of a book such as the present volume.

Part II, covering the remaining 82 pages of the text, from the pen of Dr. R. Brieger, of Berlin, deals with matters directly connected with plant analysis. A subject-matter index is appended.

We look forward with interest to the publication of the complete work.

ARTHUR R. LING.

THE CHEMISTS' YEAR BOOK, 1931. Edited by F. W. ATACK, M.Sc.Tech., D.Sc., assisted by R. T. ELWORTHY, Ph.D., F.I.C. American data in collaboration with FRANCIS M. TURNER, Jnr. Pp. 1186 and Index. Manchester: Sherratt and Hughes. Price 21s.

When the last review of this important work appeared in *THE ANALYST* (1929, 54, 563), the reviewer (F. W. F. Arnaud) commented on the inadequacy of a sixteen page index for a book of such length and scope. The 1931 edition has precisely the same number of pages as that reviewed by Mr. Arnaud—and an index of exactly the same length. There are other respects, too, in which the editor seems to have been unduly fettered by traditions that might well be allowed to atrophy.

For example, the actual format of these year-books is a morphological anachronism. Originally the *Chemiker-Kalender*, perhaps the prototype of them all, was a slim volume that any chemist might slip into his pocket before attending a scientific meeting or a court of law or even, if sufficiently enthusiastic, as a companion for a country walk. Gradually the book became fatter, a change attributable rather to the progress of science than to advancing years as such. Ultimately it had to be split—for it had become so bulky as to be impossible not only for the pocket, but also for the hand. Now it has actually reached the three volume stage. It would be just possible to distribute all three through the capacious pockets of a sports jacket, but any pretence that the volumes are "pocket-books" might surely be abandoned. How much the more so with *The Chemists' Year Book*, which sturdily remains in one volume. Surely the extra cost of resetting some of the standing tables, which would probably be necessitated by a change from demy 18mo (4 by 6 inches) to crown 8vo ( $5\frac{1}{2}$  by  $7\frac{1}{2}$  inches, the usual size of books of logarithm tables), would be more than offset by the increased convenience of handling and storing, and the consequent increased sales.

The Year Book, as before, covers an immense circle of scientific knowledge. Some of the obscurer circumferential points may be cited as witness to its range; the percentage of manganese oxide in the sand and loam of Oklahoma; the confirmatory tests for the presence of *B. coli* in water; the method of making Verde Italiano; the specific gravity of a 7 per cent. (weight in weight) solution of potassium bromate in water; the correction for pressure of the boiling point of triphenyl methane; the mean molecular weight of the fatty acids of Menhaden oil; the meaning of the terms "anthelmintic" and "taeniafuge"; the relative extensiveness of the official examinations of patents for novelty in U.S.A. and Germany; the formula relating pH and voltage for the quinhydrone electrode; and the date of Hjelms' isolation of molybdenum.

There seems to be no branch of analytical or practical chemistry that does not receive some kind of treatment in the Year Book. The editor has, indeed, compressed an encyclopædia into a tabloid volume. It may, therefore, seem ungracious to call attention to minor defects, but there is a special reason for doing so in this instance.

Generally speaking, the information in it on any particular subject will not be used by the expert in that subject, who has, besides his own special knowledge, various text-books and monographs available. Yet the dairy expert, for example, having suddenly to use a bomb calorimeter, might find the Year Book to contain just the information he needed. Precision of detail is an absolute essential in such cases.

It is obviously impossible to examine the Year Book exhaustively for accuracy on many points, even if one had the necessary knowledge; all one can do is a little random sampling. Near the top of p. 717 there is a blank space, where, presumably, the abbreviation "° C." has been omitted. Beneath the diagram on p. 721 the name Reichert is mis-spelt. Near the top of p. 730 occurs the word "gasimetric."



On pages 826 and 827 we find indiscriminate use of "grm.", "gram", "gm."—two of them wasting valuable space. The Rose-Gottlieb method for fat is not applicable with accuracy to milk powder if this is at all "stale." The industrial importance of casein would seem to warrant some reference to its analysis.

Dr. Attack has collected a brilliant and expert group of collaborators; with rather more attention to the mechanical side of the editing, and with the change of format, suggested above, in the 1933 edition (it is, presumably, too late to consider it for the seventeenth edition of 1932), *The Chemists' Year Book* should be as near perfect as almost superhuman effort can make it.

A. L. BACHARACH.

## Publications Received.

UNTERSUCHUNG DES WASSERS. By A. SPLITTBERGER and E. NOLTE. Abderhalden; Handbuch der biologischen Arbeitsmethoden. Lief. 350, 352, 358. Berlin: Urban and Schwarzenberg. Price R.M. 11 each part.

THE SOIL AND THE MICROBE. By S. A. WAKSMAN and R. L. STARKEY. London: Chapman & Hall. Price 17s. 6d. net.

SOLVENTS. By T. H. DURRANS. 2nd Edition. London: Chapman & Hall. Price 10s. 6d. net.

RECENT APPLICATIONS IN MICROSCOPY. Edited by A. PINEY. London: J. & A. Churchill. Price 12s. 6d.

DAIRY BACTERIOLOGY. By Prof. ORLA-JENSEN. Translated by P. S. ARUP. London: J. & A. Churchill. Price 18s.

BUTTER FAT (GHEE). By N. N. GODBOLE and SADGOPAL. Benares Hindu University.

KINGSTON'S EQUIVALENT TABLES (per pound and per kilogram, and per yard and per metre). Giving Equivalent Costs for British and Foreign Currencies. West India House, E.C.3. Price 2s. net each.

DIE MASSANALYSE—ZWEITER TEIL: DIE PRAXIS DER MASSANALYSE. By I. M. KOLTHOFF. Berlin: J. Springer. Price R.M. 28.

PRÄTIKUM DER WARENKUNDE. By E. GRÜNSTEIDL. Vienna: J. Springer. Price R.M. 11.50.

CHEMISTRY IN THE SERVICE OF MAN. By A. FINDLAY. 4th Edition. London: Longmans, Green & Co., Ltd. Price 6s. net.



# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

### The Examination of Dyed Leather in Cases of Alleged Dermatitis.

By T. CALLAN, M.Sc., Ph.D., F.I.C., AND N. STRAFFORD, M.Sc., A.I.C.

(Read at the Meeting, October 7, 1931.)

INTRODUCTION.—Published results of investigations of dermatitis caused by articles of attire have been concerned almost exclusively with furs, which commonly are dyed with organic bases oxidised *in situ* (cf. ANALYST, 1923, 48, 282, 283, 284; 1929, 54, 694).

Recently, however, there have been several cases of dermatitis alleged to have been caused by leather hat-bands. Investigation of authenticated cases has revealed the fact that the hat-bands in question contained small but appreciable amounts of soluble chromates or chromic acid—the results of faulty after-chroming, that is, a treatment with a dichromate to which dyed leathers are frequently subjected to improve shade and fastness. It is well known that contact of such substances with the skin is liable to cause dermatitis.

The usual dyestuffs for dyeing leather hat-bands are basic colours, such as chrysoidine and Bismarck brown. Since these are derivatives of *meta*-diamines, e.g. *meta*-phenylenediamine or *meta*-toluylenediamine, suspicion has been aroused that, possibly, traces of diamines in the dyestuffs used may also be contributory factors in cases of dermatitis alleged to be caused by dyed leather.

*p*-Phenylene-diamine is not a constituent of dyestuffs normally employed in colouring leather; nevertheless, it is our experience that, when investigating cases of dermatitis, analysts almost invariably suspect the presence of this substance, and we have known cases of its having been reported, even when the dyestuffs used were derived solely from *meta*-diamines.

It was, therefore, considered of importance to devise tests which would indicate the presence or absence of both *meta*- and *para*-diamines in the dyed leather.

Cox (ANALYST, 1929, 54, 694) has published valuable methods for the detection of diamines and allied bodies in fur.\* In the first place, therefore, the possibility

\* Since the present investigation was carried out Forster and Soyka (J. Soc. Dyers and Col., 1931, 47, 99; ANALYST, 1931, 476) have published similar methods.

of applying these methods directly to dyed leather was investigated. When they were applied both to undyed and to dyed vegetable-tanned leather, it soon became evident that the tannins present in the leather may interfere very seriously with many of Cox's tests, with the result that, unless great caution were taken in interpreting the results, it would be quite possible for the apparent presence of diamines to be indicated, even in an undyed vegetable-tanned leather, whilst in the case of a dyed leather it would be impossible to say with most of these reactions whether they were those of tannin matter or of diamines. We have reason to believe that in several cases where vegetable-tanned leather hat-bands have been reported to contain diamines, the reactions on which this conclusion was based were due to the normally-present tanning materials.

EXPERIMENTAL.—The samples of hat-band leather which we examined were as follows:—

- A. Undyed leather which had been tanned with vegetable-tanning materials.
- B. Trade-dressed leather tanned as in A and dyed with chrysoidine (a derivative of *meta*-toluylenediamine) shaded with magenta and methylene blue.
- C. Trade-dressed leather tanned as in A and dyed with basic leather phosphine shaded with malachite green, magenta and auramine. None of these dyes is a derivative of a *meta*- or *para*-diamine.
- D. Vegetable-tanned leather\* dyed with Bismarck brown (a derivative of *meta*-toluylenediamine).

The leathers were free from grease. In each case 10 grms. of the leather (cut into small pieces) were extracted for 48 hours in the cold with 40 ml. of 1 per cent. acetic acid. The extracts were then examined by the reactions given by Cox (*loc. cit.*) and by the following additional reagents: aniline and dichromate (indamine reaction); sodium hypochlorite; diazobenzene-*p*-sulphonic acid.

In order to obtain a measure of the reliability and sensitivity of the various tests the reagents were also added to portions of each leather extract, to which had been added a small amount of *para*-phenylenediamine and *meta*-toluylenediamine respectively (equivalent to 0.007 per cent. in the total solution tested, or 0.04 per cent., calculated on the original weight of leather). Control tests were also carried out on solutions containing 0.007 per cent. of *para*-phenylenediamine, 0.007 per cent. of *meta*-phenylenediamine and 0.007 per cent. of *meta*-toluylenediamine, respectively.

In addition, the approximate limiting sensitivity of certain of the tests was determined by reducing the amount of diamine added to the extract.

The following are brief notes on the tests employed:—

REAGENTS REQUIRED.—2 *N* hydrochloric acid solution; *N*/2 sodium nitrite solution; approx. *N*/20  $\beta$ -naphthol solution (0.72 gm. of  $\beta$ -naphthol is dissolved in 6 ml. of *N* sodium hydroxide solution and diluted to 100 ml.); bromine water; *N*/10 sodium hydroxide solution; dilute sodium hypochlorite solution (5 ml. of

\* This leather was of different origin from that employed in tests A to C.

commercial hypochlorite liquor containing 18-20 per cent. of available chlorine, diluted to 100 ml.); 5 per cent. aqueous solution of phenol; 1 per cent. alcoholic solution of *p*-dimethylaminobenzaldehyde; 5 per cent. ferric chloride solution; 1 per cent. aniline hydrochloride solution (1 grm. of aniline hydrochloride crystals dissolved in water and made up to 100 ml.); 2 per cent. potassium dichromate solution; sodium acetate crystals (pure); *N*/20 diazobenzene-*p*-sulphonic acid.

The last reagent is prepared by dissolving 8.65 grms. of purified anhydrous sulphanilic acid in warm water containing sufficient sodium carbonate to make the final solution slightly alkaline to litmus, cooling the solution, and making up to 100 ml. Ten ml. of this solution are pipetted into a 100 ml. beaker containing a few small pieces of washed ice and diluted to about 30 ml. Seven or eight ml. of hydrochloric acid (sp. gr. 1.16) are added while the beaker is cooled externally with ice, and then, rapidly, 10.1 ml. of *N*/2 sodium nitrite solution, previously diluted with an equal volume of ice-cold water. The whole is thoroughly mixed and diluted to 100 ml. with ice-cold water. The diazo solution should be used within an hour of preparation.

**DETAILS OF TESTS.**—In each case the reagents were added in the order named to (a) 1 ml. of the extract and 0.5 ml. of water, (b) 1 ml. of the extract and 0.5 ml. of 0.02 per cent. diamine solution.

**COX'S TESTS.**

No. 1. *Nitrous Acid.* 0.2 ml. of 2 *N* hydrochloric acid, 0.5 ml. of *N*/2 sodium nitrite solution.

No. 2. The solution from No. 1, after standing for two minutes in the cold, is added to a mixture of 4 ml. of the *N*/20  $\beta$ -naphthol solution and 1 ml. of sodium hydroxide solution.

No. 3. *Bromine.* Bromine water is added, drop by drop.

No. 4. *Phenol and hypochlorite.* *N*/10 sodium hydroxide solution, sufficient to make the solution very faintly alkaline to Brilliant Yellow paper, 1 ml. of 5 per cent. phenol solution, 2 to 5 drops of dilute sodium hypochlorite solution.

No. 5. *p*-Dimethylaminobenzaldehyde. 0.2 ml. 2 *N* hydrochloric acid, 1 ml. of the reagent.

No. 6. *Ferric chloride.* One drop of the ferric chloride solution.

**Additional tests.**

No. 7. *Aniline and dichromate.* 0.5 ml. of 1 per cent. aniline hydrochloride solution, 1 drop of 2 per cent. potassium dichromate solution.

No. 8. *Sodium hypochlorite.* *N*/10 sodium hydroxide solution, sufficient to give a faintly alkaline reaction to Brilliant Yellow paper, 1 ml. of dilute sodium hypochlorite solution.

No. 9. *Diazobenzene-p-sulphonic acid.* One grm. of pure sodium acetate crystals, 1 ml. of *N*/20 diazobenzene-*p*-sulphonic acid solution.

The results are given in the accompanying tables.

TABLE I. UNDYED VEGETABLE-TANNED LEATHER A.

Test.	Extract from leather.* (X.)	X + <i>m</i> -Tolylene-diamine equiv. to 7 parts per 100,000 of soln. or 0.04 per cent. on the leather	<i>p</i> -Phenylene-diamine equiv. to 7 parts per 100,000 of solution.	<i>m</i> -Tolylene-diamine (7 parts per 100,000).	<i>m</i> -Phenylene-diamine (7 parts per 100,000).	Conclusions.
1. Sodium nitrite.	Deep yellowish-brown.	Deep yellowish-brown.	Deep yellowish-brown.	Yellow.	Yellow.	Tannins interfere seriously.
2. Diazotised soln. from (1) + excess alkaline $\beta$ -naphthol.	Deep brown.	Deep orange-brown.	Deep brownish-orange.	Brownish-red.	Brownish-red.	Tannins interfere seriously.
3. Bromine water.	No precipitate.**	No precipitate.				Tannins interfere.
4. Phenol and hypochlorite.	Slight darkening.	Slight darkening. (Rather more than X.)				Tannins interfere (by masking).
5. <i>p</i> -Dimethyl-amino-benzaldehyde.	Pale yellow, very slowly turning orange and finally red.	Deep orange-red immediately (0.01% on leather, pale orange).	Deep yellow immediately, very slowly turning orange and finally red.	Deep yellow immediately.	Deep yellow immediately.	Tannins slowly develop a red colour which might be mistaken for an indication of the presence of <i>p</i> -diamine. Tannins interfere seriously.
6. Ferric chloride.	Immediate intense violet-blue.	Immediate intense violet-blue.				Tannins interfere.
7. Aniline hydrochloride and dichromate.	Yellowish-brown.	Yellowish-brown.				Tannins present in sufficient quantity to interfere seriously with reaction for <i>p</i> -diamine by masking. This test is much less sensitive than the others. Tannins do, however, apparently interfere (by masking).
8. Hypochlorite.	No obvious change. No precipitate.	No obvious change. No precipitate.				Tannins present in sufficient quantity to interfere seriously with reaction for <i>p</i> -diamine by masking. This test is much less sensitive than the others. Tannins do, however, apparently interfere (by masking).

TABLE II. DYED LEATHER B.

Test.	Extract from leather.* (X.)	X + <i>p</i> -Phenylenediamine equiv. 60.7 parts per 100,000 of sub. 0.04 per cent. on the leather.	X + <i>m</i> -Toluylenediamine equiv. 60.7 parts per 100,000 of solution.	<i>p</i> -Phenylenediamine (7 parts per 100,000).	<i>m</i> -Toluylenediamine (7 parts per 100,000).	<i>m</i> -Phenylenediamine (7 parts per 100,000).	Conclusions.
1. Sodium nitrite.	Slight darkening.	—	Darkens rather more than X.	Pale yellow.	Yellow.	Yellow.	Test not sufficiently sensitive to allow of any definite conclusions being drawn. (Colour of the extract also interferes.)
2. Diazotised soln. from (1) + excess alkaline $\beta$ -naphthol.	Brownish - yellow.	—	Orange-red (0.02% on leather; brownish-orange).	Reddish-brown.	Brownish-red.	Brownish-red.	No diamines ( <i>meta</i> or <i>para</i> ) in the leather.
5. <i>p</i> -Dimethylamino - benzaldehyde.	Yellowish-brown (slight darkening only).	Deep orange-red immediately (0.01% on leather; distinct reddish-orange immediately).	Deep yellow (considerably deeper than X.)	Deep orange-red immediately.	Deep yellow immediately.	Deep yellow immediately.	Leather is free from <i>p</i> -diamine and probably also from <i>m</i> -diamine.
6. Ferric chloride.	Immediate greenish - blue coloration.	—	—	Immediate darkening; changes very slowly to pale brownish-violet.	Slight darkening.	Slight darkening.	Owing to the known interference of tannins, no conclusions can be drawn.
7. Anilinehydrochloride and dichromate.	Yellow colour. (Practically no change.)	Immediate blue-green (0.01% on leather; faint olive-green, fading rapidly).	—	Immediate blue-green, rapidly turning blue.	No change.	No change (turns brown very slowly).	Leather contains no <i>p</i> -diamine.
9. Diazo - benzene- <i>p</i> -sulphonie acid.	Deep yellowish-brown.	—	Deep brownish-red (0.02% on leather; deep reddish-brown).	Light orange-brown.	Reddish-brown with precipitation.	Deep reddish-brown (with precipitation).	Leather contains no <i>m</i> -diamine.

\* Note: The extract itself was light brown in colour.

TABLE III. DYED LEATHER C.

Test.	Extract from leather.* (X.)	X + <i>p</i> -Phenylenediamine equiv. to 7 parts per 100,000 of soln. or 0.04 per cent. on the leather.	X + <i>m</i> -Toluylenediamine equiv. to 7 parts per 100,000 of solution.	<i>p</i> -Phenylenediamine (7 parts per 100,000).	<i>m</i> -Toluylenediamine (7 parts per 100,000).	Conclusions.
1. Sodium nitrite.	Very slight darkening.	—	Darkens more than X (yellowish colour).	Pale yellow.	Yellow.	Test not sufficiently sensitive to allow of any definite conclusion being drawn. Colour of extract interferes.
2. Diazotised soln. from (1) + excess alkaline $\beta$ -naphthol.	Yellow.	—	Brownish - red (0.02% on leather: brownish-orange).	Reddish-brown.	Brownish-red.	No diamines ( <i>meta</i> or <i>para</i> ) in the leather.
5. <i>p</i> -Dimethylamino-benzaldehyde.	Very slight deepening of yellow colour (no red).	Very definite orange-red immediately (0.01% on leather distinct red: dish-orange immediately).	—	Deep orange-red immediately.	Deep yellow immediately.	Leather is free from <i>para</i> -diamine and probably also from <i>meta</i> -diamine.
6. Ferric chloride.	Immediate greenish-blue.	—	—	Immediate darkening changes very slowly to pale brownish-violet.	Slight darkening.	Owing to the known interference of tannins, no conclusions can be drawn.
7. Aniline hydrochloride and dichromate.	Yellow colour. (Practically no change.)	Immediate blue-green (0.01% on leather: faint olive green fading rapidly).	—	Immediate blue-green rapidly turning blue.	No change.	Leather contains no <i>p</i> -diamine.
9. Diazo-benzene- <i>p</i> -sulphonic acid.	Golden-yellow.	—	Brownish-red (slight turbidity).	Light orange-brown.	Deep reddish-brown (with precipitation).	Leather contains no <i>m</i> -diamine.

\* Note: The extract itself was yellow in colour.

TABLE IV. DYED LEATHER D.

Test.	Extract from leather.* (X.)	X + <i>m</i> -Toluylenediamine equiv. to 7 parts per 100,000 of soln.	<i>p</i> -Phenylenediamine (7 parts per 100,000).	<i>m</i> -Toluylenediamine (7 parts per 100,000).	<i>m</i> -Phenylenediamine (7 parts per 100,000).	Conclusions.
1. Sodium nitrite.	Slight darkening.	Slight darkening.	Pale yellow.	Yellow.	Yellow.	Test not sufficiently sensitive to detect the presence of added diamine. Colour of extract also interferes.
2. Diazo-tised soln. from (1) + excess alkalineβ-naphthol.	Brownish - yellow.	Brownish-red.	Reddish-brown.	Brownish-red.	Brownish-red.	It is easily possible to detect the presence of added <i>meta</i> -diamine. Colour of extract does not cause serious interference. Leather gives no reaction for <i>meta</i> -diamine.
5. <i>p</i> -Dimethyl-amino-benzaldehyde.	Yellow (colour of reagent).	Deep yellow immediately.	Deep orange-red immediately.	Deep yellow immediately.	Deep yellow immediately.	Colour of extract does not seriously interfere. It is easily possible to detect the presence of added diamine. The leather gives no reaction for <i>meta</i> -diamine.
9. Diazo-benzene- <i>p</i> -sulphonic acid.	Orange-yellow.	Orange-red.	Light orange-brown.	Reddish-brown (with reddish ppt.).	Deep reddish-brown (with precipitation).	It is easily possible (by direct comparison) to detect the presence of added <i>meta</i> -diamines. The leather gives no reaction for <i>meta</i> -diamine.

\* Note: The extract itself was pale reddish-brown in colour.



**DISCUSSION OF RESULTS.**—From the results given in Table I, it is evident that the tannins present in the leather may interfere with many of the colour reactions. In some cases the interference is positive; that is, colour reactions are obtained which might easily be mistaken for those given by diamines (Tests 1, 2, 5, 6 and 9). In others, the effect of tannins is to mask, to a greater or less extent, the effect produced by diamines when these are present (Tests 3, 4, 7 and 8).

It is evident, therefore, that very considerable caution is required in interpreting the results of the tests.

From Tables II, III and IV it is noticeable that the extracts from the dyed leathers give much less pronounced reactions with the various reagents than the extract from the tanned but undyed leather. This, of course, is to be ascribed to the fact that a proportion of the soluble tannins has been removed by the dyeing process. Consequently, in certain tests, particularly 5 and 7, the disturbing effect of the tannin reaction is much less noticeable.

By a comparison of the reactions given by the extracts of the dyed leathers with those given by the extracts containing very small amounts of *meta*-toluylenediamine and *para*-phenylenediamine, it is possible to arrive with a considerable degree of accuracy at a conclusion as to the presence or absence of traces of diamines.

Actually in the three samples of dyed leather referred to in this paper no diamines could be detected.

**RECOMMENDATIONS.**—Only the tests numbered 2, 5, 6, 7 and 9 should be applied to the extracts from leather which is being examined for the presence of diamines. No. 5 is the most sensitive of the tests, and will detect as little as 0.01 per cent. *p*-phenylenediamine (expressed on the leather) in a coloured extract containing tannins. Test 7 will detect a similar amount, but only if test 6 indicates that more than traces of tannin are present. Tests 2, 5 and 9 will detect about 0.02 per cent. of a *meta*-diamine in a similar extract. This limiting sensitivity of the tests is largely determined by the presence of tannins (indicated by test 6) and colouring material from the leather. The sensitivity of the tests is considerably greater when the reactions are carried out in the absence of these interfering factors.

In view of the fact that the presence of tannins may lead to faulty conclusions being drawn even from these tests, an examination of the extract should always be supplemented by control tests on portions of the extract to which very small amounts of a *meta*- and *para*-diamine, respectively, have been added.

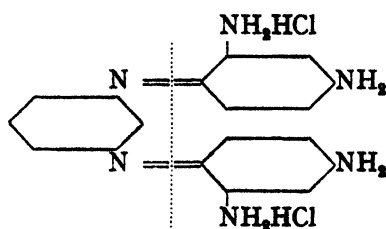
In this way the effect produced by the diamines may be noted with accuracy, and from a careful survey of the whole of the evidence given by the four tests one may draw reliable conclusions as to the presence or absence of diamines in leather.

In conclusion, we wish to express our thanks to Imperial Chemical Industries, Ltd., Dyestuffs Group, for permission to publish the results obtained, and also to Mr. R. T. Parry-Jones for his helpful assistance.

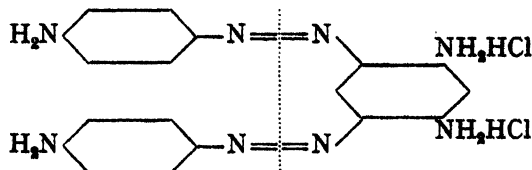
## DISCUSSION.

Dr. H. E. Cox said that the authors had drawn attention to a point which experience showed to be very important; he could quite confirm their findings as to the interference of leather extract or tannin materials. It was very necessary, before making tests by colour reactions, to ascertain what other substances were present and the extent of their possible interference. In his paper, to which the authors had referred, he had given reactions applicable to the pure intermediates, and these were quoted and confirmed by Dr. Callan and Mr. Strafford. He had been particular to indicate that the extracts should be made from the hair and not usually from the fur as a whole. From this point of view furs were quite different from other dyed articles, in that the fibre was impregnated with the appropriate intermediate, which was subsequently oxidised on the fibre; traces of residual or unoxidised bases were easily leached or soaked out.

There was another point which was liable to introduce serious error which the authors had not mentioned; indeed, it did not appear in any of the literature, so far as he knew, that was the decomposition of either oxidation colours or various azo colours during extraction. To take the simplest case, one might consider Bismarck brown, the formula of which was well known to be:



It was made from meta-phenylenediamine and nitrite, and, on reduction with zinc dust or hydrosulphite, split at the double bond, re-forming metaphenylenediamine. He had established experimentally that digestion of an animal tissue, such as leather, with hot dilute acid effected the same reduction, differing only in degree. In this way it was possible to form by an inappropriate technique one of the substances sought for and so arrive at an erroneous conclusion. This was a simple case. Then, to consider a more complex one, there was a well-known brown leather dye having the constitution (according to the Colour Index)



it was made from para-aminoacetanilide and meta-phenylenediamine. On reduction it also split at the double bond, and there were formed two molecules of para-phenylenediamine, which substance was not only not present in the dye, but was not even one of the constituents or ingredients from which the colour was made. He thought that the non-recognition of such facts might explain some difficult results. It was within his own experience, which now extended over some hundreds of specimens, that substances had been occasionally suggested as being present which could not really be there, having regard to the dyes or materials used by the manufacturers.

A similar point arose with oxidation products where the pigment was a complex ring structure, often of imperfectly known constitution; these might be readily decomposed in acid solution with protein matter and free base re-formed. Indeed, one theory of dyeing presupposed the formation of complex but loose compounds with animal bases, and their hydrolytic or reduction products were liable to be misleading unless the possibilities were borne in mind.

He rather mistrusted the indamine reaction because so very many compounds would give it, but every possible test should be applied in these difficult cases.

Dr. H. PHILLIPS said that the British Leather Manufacturers' Research Association had found that the testing of the actual leather extract was unsatisfactory because of the presence of tannins, and they preferred to extract the diamines from the leather extract. Benzene was used for the extraction in a continuous extractor, and for small amounts of diamines 24 hours' extraction was necessary. He had used the same extraction agent as Mr. Strafford, and had found that if semi-chrome leather were painted with a solution of diamines and then allowed to dry, it was not possible, in the case of *m*-phenylenediamine, to extract the diamine with 1 per cent. acetic acid, although it could be extracted with *N*/10 hydrochloric acid. In testing for *p*- and *m*-phenylenediamines a very useful method was to steam-distil the leather with an alkaline solution, using superheated steam. In this manner a colourless distillate was obtained which was very satisfactory for the colour reactions. This method, however, was only suitable for preliminary examination, since, under these conditions, diamines might be generated from normally stable dyes.

Mr. Strafford had mentioned hat-band leathers as a possible cause of dermatitis, owing to the presence of chromates. The greater proportion of hat-bands were made of vegetable-tanned leathers. In experiments which he had made he had been surprised to find that vegetable-tanned leather would fix up to 10 per cent. of its weight of chromate. The presence of chromate in semi-chrome hat-band leather was unlikely to be the cause of dermatitis. Most semi-chrome leathers yielded traces of chromate when extracted with water, and no suspicion was attached to such leathers.

Dr. W. L. DAVIES remarked that, since chromium oxide had been successfully used as a "clue substance" in determining the digestibility of foods, it could not be regarded as an internal poison. Also, did not chromium poisoning depend largely on the individual, one person being affected, whilst another was immune?

Mr. STRAFFORD said that he would like to express his thanks to Dr. Cox for his very valuable comments. Regarding the indamine test there was no doubt that the most important factor controlling the sensitivity of the reaction was the degree of acidity of the solution. Whilst he agreed that the test might not be specific for *para*-phenylenediamine he considered it of value, since in coming to a decision one was guided by the whole of the evidence from the various tests and not by the result of one reaction only. Quite powerful reducing agents were required to reduce azo dyestuffs to amino compounds, and he was surprised to hear the suggestion that leather could act as a sufficiently powerful reducing agent to produce compounds such as *meta*- or *para*-diamines from certain types of azo dyestuffs. This was, of course, theoretically possible with dyestuffs of the constitution outlined by Dr. Cox.

Replying to Dr. Phillips' remarks, Mr. Strafford agreed that the use of superheated steam was rather open to question, since this might cause decomposition of the dyestuff. Acetic acid was chosen for the extraction, following the precedent set by Dr. Cox in the case of dyed furs. The use of this acid more closely conformed to the conditions likely to be met in cases of alleged dermatitis than hydrochloric

acid. Mr. Strafford stated that he was also interested in the question of the separation of diamines from tannins. As a matter of fact, since the completion of the work now presented he had carried out a few experiments on the chemical separation of the diamines from the tannins, and he had obtained reasonably good results by making the extract from the leather distinctly alkaline and extracting with ether.

Dr. Phillips was correct in differentiating between the toxicity of chromium salts and chromates or dichromates. In the case mentioned, soluble chromates or chromic acid had been detected in the leather extract.

## The Determination of Small Quantities of Methane.

By H. R. AMBLER, B.Sc., F.I.C.

IN the following paper a convenient and precise technique is described for the determination of methane in gas mixtures; it is particularly applicable to mixtures containing small proportions of methane.

**INTRODUCTION.**—The determination of methane (and its homologues) is made difficult by its inert character and its lack of specific chemical reactions applicable to analysis. In practice, it has always been finally determined by combustion with oxygen, after removal of interfering gases. Such gases as carbon dioxide, oxygen, and unsaturated hydrocarbons are removed without difficulty; the main concern is the removal of carbon monoxide.

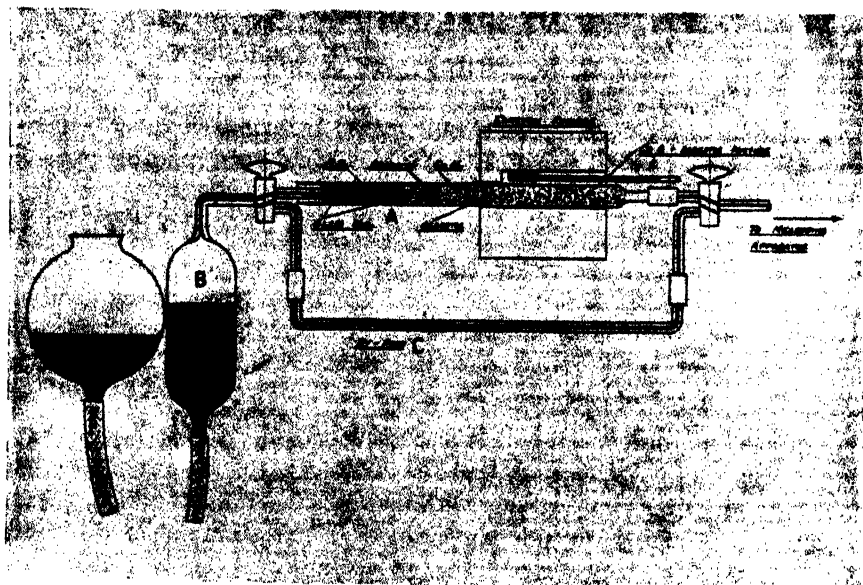
In one method, still much in use, carbon monoxide is absorbed by cuprous chloride or other liquid absorbent, and the hydrogen that is usually present is exploded with air or oxygen, together with the methane, the latter being measured by the amount of carbon dioxide so produced. The accuracy of this method, however, is limited to about 0.2 per cent. on account of incomplete absorption of carbon monoxide. (*Cf.* Ambler, *ANALYST*, 1925, 50, 167; Sutton and Ambler, *ibid.*, 172.)

In another method the bulk of the carbon monoxide is removed by absorption, and the remainder by passing over iodine pentoxide at 120°–150° C. The hydrogen and methane are then oxidised by passing over copper oxide at 950°–1000° C., the water so produced removed by phosphorus pentoxide, and the methane determined gravimetrically by absorption of the carbon dioxide in alkali. This method is accurate, but takes some hours, and requires large samples of gas, particularly where the methane content is low.

In the process here described, hydrogen and carbon monoxide are oxidised by means of cupric oxide at about 300° C. (*cf.* Jäger, *J. Gasbeleucht.*, 1898, 41, 764), the resulting carbon dioxide is removed, and the methane is determined by burning with oxygen in the presence of platinum wire at bright yellow heat.

**APPARATUS AND PROCEDURE.**—(a) *Removal of Carbon Monoxide and Hydrogen.*  
—The apparatus for the fractional combustion is shown in the diagram.

A is a glass tube, one end of which contains a mixture of powdered cupric oxide and short-fibre asbestos in equal parts.\* The other end of the tube contains calcium chloride, ascarite (a sodium hydroxide and asbestos preparation) and phosphorus pentoxide. If carbon monoxide is to be determined in the same process, the ascarite and calcium chloride are omitted. The end containing the copper oxide is surrounded by an electric furnace. The other end leads to a reservoir, B, of about 150 c.c. capacity, which can be used as a Töpler pump to evacuate the tube A; gas so pumped from A is transferred through the by-pass, C, either to the air or to the measuring part of the apparatus.



At the beginning of an experiment the furnace is heated to  $270^{\circ}$ – $295^{\circ}$  C.,† and the tube A evacuated. A measured sample of gas is drawn slowly through the copper oxide into B, and passed slowly backwards and forwards three times. The gas remaining in the tube is now pumped out and returned through C to a measuring apparatus.

The gas now consists of nitrogen, together with small quantities of methane or its homologues. If the nitrogen content of the original gas is known, it is

\* This exposes a much greater surface than the more usual "wire" copper oxide and reduces the time required for the oxidation (see Donnelly, Foott and Reilly, *Proc. Roy. Dublin Soc.*, 1929, 19, 165).

† Methane is unaffected below  $295^{\circ}$  C. (Ott and Scherb, *Z. anal. Chem.*, 1926, 68, 238). It would appear that oxidation may in some cases begin at  $300^{\circ}$  C. (Terres and Manguin, *J. Gasbeleucht.*, 1914, 57, 8). See also Campbell and Gray, *J. Soc. Chem. Ind.*, 1930, 49, 432r.

unnecessary to measure the volume of sample taken in the first place, since the next process gives the ratio of methane to nitrogen.

(b) *Combustion of Methane*.—The residual gas is measured in a gas analysis apparatus fitted with a platinum wire combustion pipette. The apparatus in use here is one of the Ambler type (ANALYST, 1929, 54, 517), fitted with a vessel for slow combustion. The Haldane apparatus would also be suitable. A small amount of oxygen or air is let in, the gas measured again and transferred to the combustion pipette. Any carbon monoxide or hydrogen that may have escaped the copper oxide may be detected by heating the platinum wire at very dull red heat for a minute and measuring the gas again. If these gases are present they will burn under these conditions and contraction will be observed; methane is not affected at all (Whitaker, *Fuel*, 1925, 4, 450). Only in exceptional cases is such contraction greater than 0.1 per cent. on the residual gas.

The gas is now treated with the platinum at bright yellow heat for two minutes, and the volume again measured. Combustion of one volume of methane causes a contraction of two volumes. The gas so burnt can be identified as methane by measuring the amounts of carbon dioxide produced and oxygen remaining. The combustion of ethane gives different ratios, and it can thus be detected and determined by measuring these quantities.

ACCURACY.—With the measuring apparatus used here a contraction of 0.05 per cent. corresponds with a pressure drop on the manometer of about 0.5 mm. This is easily readable, and would be produced by the combustion of 0.025 per cent. of methane in the gases remaining after removal of carbon monoxide and hydrogen, *i.e.* in the nitrogen. For gases of low nitrogen content the sensitivity of the method is increased, since the quantity of gas taken for the final combustion corresponds with a larger quantity of the original gas. If the nitrogen content is, for example, 10 per cent., 0.0025 per cent. of methane in the original sample is easily measurable.

The following duplicate determinations show the degree of concordance obtained. The nitrogen content of the gases was from 16 to 19 per cent., and the volumes of original samples about 70 c.c.:—Sample 1, 0.040 and 0.040; sample 2, 0.089 and 0.090; sample 3, 0.119 and 0.116; sample 4, 0.158 and 0.155 per cent. of methane.

RESEARCH DEPARTMENT,  
WOOLWICH.

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## The Effect of the Rate of Boiling on the Residual Sulphur Dioxide Content in Mixtures of Sugar and Corn Syrup; also the Effect of Bleaches containing Sulphur Dioxide.

By R. HAROLD MORGAN, B.Sc., A.I.C.

IN a former communication (ANALYST, 1930, 55, 488) I discussed the effect of temperature on the sulphur dioxide content of mixtures of sugar and corn syrup which had been boiled at various temperatures. It was shown that the amount of sulphur dioxide evolved during the boiling process varies with the temperature to which the mixture is raised.

Furthermore, a method was indicated by which an analyst can determine the temperature at which a sample has been boiled, and then work out the limit of sulphur dioxide which should not be exceeded, assuming the original constituents contained their maximum allowance of sulphur dioxide under the Preservatives Regulations.

At the request of the Publication Committee further work has been carried out to determine the effect of variations in the rate at which mixtures of sugar and corn syrup are boiled. The results in the former paper were obtained by rapid boiling. The required temperatures were reached in the shortest possible time, and this is in accordance with industrial practice, as any unnecessary delay in the boiling process, particularly in the case of the "higher boils," causes discoloration of the resulting product.

**EXPERIMENTAL PROCEDURE.**—The method of boiling and the analytical procedure were similar in every respect to those used in the former investigation (*loc. cit.*). Four ordinary corn syrups (or "confectioners' glucose"), as supplied to the trade, were used. The fourth series of results, *i.e.* with the corn syrup N 381, is worthy of note, as the syrup contained the maximum amount of sulphur dioxide permitted under the regulations.

The times of boiling were chosen arbitrarily, bearing in mind the minima necessary to reach the desired temperatures, as shown by previous results. In each case an endeavour was made to lengthen the time throughout the complete heating process, but if the desired temperature was reached before the time limit had expired, care was taken to maintain the boiling at the correct temperature for the remaining period.

RESULTS.—The following tables show the results obtained:—

## I.

## CORN SYRUP, B 728.

Total solids, 83 per cent.; sulphur dioxide content, 395 parts per million.

Initial sulphur dioxide content of sugar and corn syrup mixture = 103 parts per million, on dry solids.

Temperature °F.	Time in minutes.	Total solids. Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
			Found.	Average.	
250	10	90.5	54	54	60
	10	90.5	54		
	12	90.5	48	48	53
	12	90.6	48		
	14	90.6	46	46	51
	14	90.6	46		
	16	90.6	46	46	51
	16	90.5	47		
270	12	94.4	42	41	44
	12	94.3	41		
	14	94.4	35	35	37
	14	94.3	36		
	15	94.3	35	34	36
	15	94.4	34		
280	13	95.0	29	29	31
	13	95.0	30		
	15	95.0	27	27	29
	15	94.9	28		
	16	94.9	28	27	29
	16	95.0	27		
300	14	97.5	12	13	14
	14	97.3	13		
	16	97.5	7	7	8
	16	97.5	8		
	18	97.6	6	6	6
	18	97.4	6		



## II.

## CORN SYRUP, M 710.

Total solids, 83 per cent.; sulphur dioxide content, 358 parts per million.

Initial sulphur dioxide content of sugar and corn syrup mixture = 94 parts per million on dry solids.

Temperature °F.	Time in minutes.	Total solids. Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
			Found.	Average.	
250	10	90.5	61	62	66
	10	91.0	63		
	12	91.0	58	57	63
	12	90.7	56		High results.
	14	90.8	56	56	62
	14	91.0	56		
270	12	95.0	42	41	43
	12	95.0	40		
	14	94.6	35	35	37
	14	95.0	36		High results.
	15	94.6	33	34	36
	15	94.7	35		
280	13	95.7	22	22	23
	13	95.9	22		
	15	95.5	19	18	20
	15	96.0	18		
	16	95.7	18	17	18
	16	95.9	17		
300	14	97.6	9	9	9
	14	97.5	9		
	16	97.5	7	6	6
	16	97.9	6		
	18	97.9	6	6	6
	18	97.8	7		

## III.

## CORN SYRUP, B 120.

Total solids, 83 per cent.; sulphur dioxide content, 371 parts per million.

Initial sulphur dioxide content of sugar and corn syrup mixture = 97 parts per million on dry solids.

Temperature °F.	Time in minutes.	Total solids. Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
			Found.	Average.	
250	10	90.9	54	53	59
	10	90.5	53		
	12	90.7	51	51	57
	12	90.4	52		
	14	90.5	49	49	55
	14	90.7	50		
270	12	94.9	37	34	36
	12	95.0	38		
	14	94.7	36	33	35
	14	94.8	35		
	15	94.6	33	34	36
	15	94.7	35		
280	13	95.5	22	22	23
	13	95.7	23		
	15	95.7	21	20	21
	15	95.9	20		
	16	95.9	18	19	20
	16	95.5	20		
300	14	97.3	10	9	9
	14	97.4	9		
	16	97.4	6	6	6
	16	97.4	6		
	18	97.3	6	6	6
	18	97.4	6		

## IV.

Total solids, 83.5 per cent.; sulphur dioxide content, 450 parts per million.

Initial sulphur dioxide content of sugar and corn syrup mixture = 117 parts per million on dry solids.

Temperature °F.	Time in minutes.	Total solids. Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
			Found.	Average.	
250	10	90.8	81	81	89
	10	90.5	81		
	12	90.5	79	78	86
	12	90.8	78		
	14	90.7	77	77	85
	14	90.5	77		
270	12	94.7	60	60	63
	12	94.6	61		
	14	94.7	56	55	58
	14	94.8	55		
	15	94.7	54	54	57
	15	94.6	54		
280	13	95.5	36	37	39
	13	95.7	38		
	15	95.5	36	36	38
	15	95.7	36		
	16	95.5	36	35	37
	16	95.6	35		
300	14	97.8	16	17	17
	14	97.7	18		
	16	97.4	13	13	13
	16	97.5	13		
	18	97.7	11	11	11
	18	97.5	12		

From these tables it will be seen that the additional time taken over the boiling process slightly reduces the residual amount of sulphur dioxide, but that a constant figure is soon reached. With the syrup used for Table I, boiling at 250° F., a fourth trial covering a period of 16 minutes was carried out, but the result was the same as that of the 14 minutes' boiling.

The amounts of residual sulphur dioxide after prolonged boiling bear no relation to each other, taking into account the initial amount of sulphur dioxide present in the mixture. The figures vary, especially in the 250° F. and 270° F. boiling, possibly owing to changes in viscosity. The results obtained from these particular temperatures with corn syrup M 710 are definitely out of proportion when compared with results hitherto obtained. Repeated boiling and analysis

gave similar results, and a possible reason of the abnormality is that this particular corn syrup was obtained from a different source.

BLEACHES.—Only two samples of bleach were obtainable, and these came from recognised trade suppliers.

One was a liquid bleach composed of a practically saturated solution of bi-sulphite containing free sulphur dioxide. The available sulphur dioxide was 29·5 per cent. (w/v).

The makers recommended the use of  $2\frac{1}{2}$  fluid ounces per cwt., which is equivalent to adding 455 parts of sulphur dioxide per million on the dry solids. After carrying out the first series of tests with corn syrup B 728 and adding the equivalent amount, viz. 1·35 c.c., the amount of bleach was reduced in subsequent experiments to 0·6 c.c., which is roughly equivalent to 1 oz. per cwt., or an addition of 202 parts of sulphur dioxide per million on the dry solids. Owing to the strength of the bleach, even with the smaller amount, high quantities of residual sulphur dioxide were found.

# V.

## CORN SYRUP, B 728.

With liquid bleach in the recommended proportion of  $2\frac{1}{2}$  fluid ozs. per cwt. Initial sulphur dioxide content on dry solids was raised from 103 to 558 parts per million.

Temperature °F.	Time in minutes.	Total solids. Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
			Found.	Average.	
250	10	90·8	461	462	510
		90·7	464		
280	13	95·9	352	358	373
		95·9	364		
300	14	97·3	209	205	208
		97·2	202		

# VI.

## CORN SYRUP, M 710.

With liquid bleach in the proportion of 1 fl. oz. per cwt.

Initial sulphur dioxide content on dry solids was raised from 94 to 296 parts per million.

Temperature °F.	Total solids. Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
		Found.	Average.	
250	90·9	218	219	241
	91·0	221		
280	95·3	143	141	149
	95·6	140		
300	97·2	64	60	63
	97·6	57		

## VII.

## CORN SYRUP, B 120.

With liquid bleach in the proportion of 1 fl. oz. per cwt.

Initial sulphur dioxide content on dry solids was raised from 97 to 299 parts per million.

Temperature °F.	Total solids. Per Cent.	Residual sulphur dioxide parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
		Found.	Average.	
250	90.8	224	222	245
	90.9	221		
280	95.9	150	143	154
	95.9	147		
300	97.2	64	62	64
	97.3	61		

## VIII.

## CORN SYRUP, N 381.

With liquid bleach in the proportion of 1 fl. oz. per cwt.

Initial sulphur dioxide content on dry solids was raised from 117 to 318 parts per million.

Temperature °F.	Total solids Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
		Found.	Average.	
250	90.9	249	247	273
	90.7	246		
280	95.9	182	184	191
	95.7	186		
300	97.5	106	104	106
	97.7	102		

The other sample of bleach consisted of white powdered hydrosulphite with an available sulphur dioxide content of 53.4 per cent. by weight. The recommended quantity for use was 2 ozs. per cwt., but in the following experiments the equivalent of half this amount was added, *viz.* 0.5 gm. per boil. This meant the addition of 305 parts of sulphur dioxide per million calculated on the dry solids.

I understand that this powder is sold as conforming with the requirements of the Food and Drugs Adulteration Act, 1928, but, while I do not question its chemical purity, its use does not appear to conform with the spirit of the Public Health (Preservatives in Food) Regulations.

## IX.

## CORN SYRUP, B 728.

Solid bleach in proportion of 1 oz. per cwt.

Sulphur dioxide content was raised from 103 to 408 parts per million on solids.

Temperature °F.	Time in minutes.	Total solids. Per Cent.	Residual sulphur dioxide parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
			Found.	Average.	
250	10	90.4	282	280	310
		90.6	278		
280	13	95.5	205	210	219
		95.9	214		
300	14	97.1	120	116	119
		97.2	111		

## X.

## CORN SYRUP, M 710.

Solid bleach in proportion of 1 oz. per cwt.

Sulphur dioxide content was raised from 94 to 399 parts per million on solids.

Temperature °F.	Total solids. Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
		Found.	Average.	
250	90.9	269	270	299 (high)
	90.8	272		
280	95.7	180	176	184
	96.0	172		
300	97.9	94	96	99
	97.8	99		

## XI.

## CORN SYRUP, B 120.

Solid bleach in proportion of 1 oz. per cwt.

Sulphur dioxide content was raised from 97 to 402 parts per million on solids.

Temperature °F.	Total solids. Per Cent.	Residual sulphur dioxide parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
		Found.	Average.	
250	90.8	266	267	294
	90.9	269		
280	95.4	182	180	189
	95.8	179		
300	97.3	102	102	105
	97.2	102		

## XII.

## CORN SYRUP, N 381.

Solid bleach in proportion of 1 oz. per cwt.

Sulphur dioxide content raised from 117 to 422 parts per million on solids.

Temperature °F.	Total solids. Per Cent.	Residual sulphur dioxide, parts per million.		Residual SO <sub>2</sub> per 1,000,000 pts. dry solids.
		Found.	Average.	
250	90.7	282	282	312
	90.7	282		
280	95.7	192	191	200
	95.5	190		
300	97.3	120	121	125
	97.4	122		

The use of these bleaches, even in small amounts, considerably raised the residual sulphur dioxide content of the various boilings when compared with boilings to which no bleach had been added. No definite relationship can be deduced from the above figures, as, no doubt, slight variations in the application of the heat will affect the rates of decomposition of the bleaches.

The position with regard to the use of bleaches in confectionery and allied industries does not appear to be clear, since some manufacturers maintain that a limited amount of the chemicals can be used, providing that the final sulphur dioxide content is not greater than that initially present in the raw materials themselves. On the other hand, other manufacturers have eliminated the use of bleaches, and, after a considerable amount of research work, have altered their processes accordingly.

Public Analysts, while realising that a considerable proportion of the sulphur dioxide goes off during manufacturing operations, as is shown in this and my previous paper, have been unable to detect the addition of bleach excepting in those cases where the sulphur dioxide content is undoubtedly above the maximum initial limit.

It is desirable that a limit should be set for the amount of sulphur dioxide in confectionery. A limit of 100 parts per million would cover all products under this heading, as is shown from the experimental results dealing with boilings at 240° F. and upwards. Of course, such a limit as that suggested would not prevent the use of bleaching agents, but it would restrict the amount which could be added during manufacturing operations, and so define the position for the Public Analyst and form another safeguard in the interest of the general public.

## The Examination and Commercial Analysis of Cotton Cloths.

By R. H. KAY.

*(Read at the Meeting of the North of England Section, October 25, 1930.)*

A LARGE proportion of the work required in the laboratory attached to a manufacturing firm will consist in getting out particulars of cloths for quotation purposes.

Most of the "text book" methods are too long; on the other hand, some of the short methods in use are very inaccurate and may lead to serious mistakes. Hence an attempt has been made to find a method that will give reasonably accurate results in the shortest time. The results have been checked in as many ways as possible, and many hundreds of thousands of yards of cloth have been made in accordance with the particulars obtained by the method described.

**EFFECT OF MOISTURE IN THE CLOTH.**—Owing to the hygroscopic character of cotton it is practically impossible in any ordinary laboratory to make an accurate analysis of a sample of cotton material without first spending considerable time on drying or conditioning. Even then the results will depend on the prevailing temperature and humidity.

To obtain a constant weight, the Shirley Institute recommends drying over phosphoric anhydride in an evacuated desiccator; by this method it is possible to get a constant weight in 4 to 6 weeks (*J. Text. Inst.*, April, 1930).

In laboratories such as those of the Shirley Institute, some Chambers of Commerce, etc., the humidity is kept as constant as possible (usually 70 per cent.), and temperature and humidity records are kept.

When physical tests—such as strength, elasticity, etc.—are required, the sample is allowed to "condition" in this atmosphere for some time before the tests are made; the length of time required to condition properly is generally considered to be at least 6 hours.

Cotton is said to be in "correct condition" when it contains 7.834 per cent. of moisture; this is equal to a regain of  $8\frac{1}{2}$  per cent. on the dry weight. In yarn and cloth analysis it is a usual custom to dry the sample at 105° C. and to calculate the correct condition weight by allowing  $8\frac{1}{2}$  per cent. regain on the dry weight. In actual practice it is quite unusual to find a sample that is in "correct condition." The patterns received for analysis are in practically any condition, and it is quite usual to get one that has been carried about in someone's pocket for an hour or so.

Heavily-sized cloths naturally contain excess moisture, but this is reckoned as part of the size. Ordinary grey cloth, containing 10–20 per cent. of size on the warp, rarely contains excess moisture, and in most cases it is bought and sold



without any special moisture content being specified. The patterns received are generally tested as soon as possible, and the results are handed in as referring to "in condition received."

**ANALYSIS OF GREY CLOTH.**—As a description of the methods of analysis for all the various cloths passing through the laboratory would occupy too much space, this paper is confined to the method used for an ordinary grey (*i.e.* unbleached and unfinished) cloth.

The particulars of a grey cloth required for quotation are:—Weave; warp threads per inch; weft threads per inch; count of warp; percentage of size on warp; count of weft; weight of cloth for stated width and length. It is also a help to know the percentage of size on the cloth.

*Weave.*—This is the way in which the threads are interlaced—plain, twill, sateen, drill, percale, poplin, leno, etc., etc.

*Warp Threads per Inch.*—The warp threads are those that run parallel along the *length* of the piece; it is usual to count in several places if the pattern is large enough, and it is not usual to find much variation, as the threads are accurately spaced by the healds and reed of the loom. Occasionally a thick place is found, and, if the pattern is a very small one and the thick place comes in the portion that has to be weighed, the calculated weight of the cloth will be on the heavy side.

A sized warp thread can usually be detected at once by testing with iodine, since the size nearly always contains starch.

*Weft Threads per Inch.*—Weft threads are those that run across the cloth from edge to edge. When the thread has been drawn across the cloth by the shuttle it is "beaten up" to the preceding thread before the shuttle comes back again.

This beating up is sometimes uneven, and, for this reason, it is quite usual to find a considerable variation in the number of weft threads per inch; in such a case the only thing to do is to count the threads in as many places as possible and to take an average.

*Count of Warp and Weft.*—The count of a yarn is another term for the size or thickness of the thread. Roughly speaking, there are two systems of fixing the count:—(1) *Fixed weight systems*, in which the number of hanks, leas, cuts, or some other definite measure of length, required to weigh 1 lb., or some other fixed weight, determines the count; *i.e.* the higher the count number, the finer the yarn; and (2) *fixed length systems*, in which the unit of length is fixed, and the count number is determined by the weight of that unit length, *i.e.* the coarser the yarn, the higher the count.

Cotton belongs to the first group, and the count is based on the number of hanks of 840 yards that are required to weigh 1 lb. For example, a 16<sup>s</sup> yarn means that 16 hanks of 840 yards weigh 1 lb.; that is, there are 16 × 840 or 13,440 yards of this particular yarn in 1 lb. A 16<sup>s</sup> yarn is twice as heavy as a 32<sup>s</sup> yarn, and so on.

Unfortunately there are different systems of fixing the count for different materials. Linen and wool belong to the same group as cotton, but the count is based for linen on the number of hanks of 300 yards that are required to weigh 1 lb., for wool on the number of skeins of 256 yards, and for worsted on the number of skeins of 560 yards required to weigh 1 lb. Jute, silk and rayon belong to the second group, *i.e.* with a fixed length and a varying weight. For jute the count is based on the weight of a spindle of 14,400 yards; *i.e.* a  $4\frac{1}{2}$  lb. jute means that 14,400 yards weigh  $4\frac{1}{2}$  lbs., and a 9 lb. jute would be twice as heavy (or thick) as the  $4\frac{1}{2}$  lb. yarn. For silk and rayon the count\* is based on the weight of a skein of 476 metres in deniers.†

When testing the count of cotton yarn it is customary to reel off a number of small hanks of 120 yards; these are called "leas," and are  $\frac{1}{7}$ th of a hank proper. They are weighed to the nearest  $\frac{1}{4}$  grain and the count calculated. At least six tests should be made, more if possible, and the results averaged. For instance, twelve tests made on a sample of 18<sup>s</sup> yarn may show a variation of  $17\frac{1}{2}$  to  $18\frac{1}{2}$ , or even more, but the average may be 18·1, or thereabouts. This result may be inaccurate unless the yarn has been previously conditioned in an atmosphere that will give a moisture content of about  $7\frac{1}{2}$  per cent., or the moisture has been determined and the count calculated "in correct condition weight."

When the count of a yarn is found by weighing a few inches or yards, more often than not a figure representing a half or quarter count may be obtained. For example, the count of a yarn may be found to be  $43\frac{1}{2}$ , but as no such uneven count is spun, it can only be inferred that it is 42<sup>s</sup> or 44<sup>s</sup>. If size has been removed by "boiling out," it will probably be 42<sup>s</sup>, as there is always a slight loss on boiling out.

*Size on Warp.*—Practically all single warps are sized to enable the threads to withstand the strain and friction of weaving. There are many special sizing preparations on the market, but they mostly consist of starchy material which has been rendered more soluble by previous treatment (such as "quellin," dextrin, "textiline," "frog starch"), together with tallow substitute—often nothing but soap—or sulphonated tallows. Gums and waxes, such as tragacanth, spermaceti, paraffin wax, etc., may also be used, and china clay is often present.

For weaving the percentage of size is always calculated as gain on 100; *i.e.* 20 per cent. of size means that 100 lbs. of yarn, when sized, will weigh 120 lbs., not that 100 lbs. of sized yarn contain 20 lbs. of size and 80 lbs. of yarn.

In heavily-sized goods one or more of the following salts are usually present:—magnesium chloride, zinc chloride, magnesium sulphate, sodium sulphate, and, possibly, calcium chloride or barium sulphate. Zinc chloride is generally used with "anti" (magnesium chloride) to prevent the growth of mould. The newest antiseptic is salicylanilide, sold under the name of "Shirlan."

\* A comparison may be of interest:—8<sup>s</sup> cotton = 22·4<sup>s</sup> linen; = 26·25<sup>s</sup> woollen; = 12<sup>s</sup> worsted; = 2·14 lbs. jute; = 660 denier silk or rayon.

† A denier = 0·0531 grm.

*Weight of Cloth.*—This is usually given in lbs. (so many lbs. for, say, 36" wide by 100 yards long). Sometimes the weight may be required in kilos. for so many cm. wide by so many metres long.

The cloth is usually measured and weighed at the mill, and the weight is generally given to the nearest  $\frac{1}{4}$  lb.

Here, again, absolute accuracy is practically impossible, as the weight of any particular piece will vary from time to time according to the humidity and temperature of the place it happens to be stored in.

**METHOD OF ANALYSIS.**—The following is an outline of the method used for an ordinary grey cloth, made of single yarns and containing a sized warp. The lengths of yarn and dimensions of pattern given below are for guidance only and will, of course, vary with the size of the sample submitted.

1. Count the number of warp threads and weft threads per inch.
2. With a dissecting needle tease out sufficient warp and weft threads to give 4 yards of each. These should be made into small bundles for cutting to the desired length. For example, take 4 lots of 9 threads and trim each little bundle to exactly 4 inches. Before cutting, stretch the threads sufficiently taut to pull out the waviness introduced by weaving, but do not stretch as "tight as possible." The heavier the yarn the fewer the number of threads that should be cut together. To distinguish between warp and weft tie the warp bundle together with a single thread and loosely knot the weft bundle. Note whether the weft is spun "twist way" or "weft way."
3. Trim a piece of the cloth to form a square, 4" by 4". The use of a template is not recommended; it is better to pull out the side threads until the desired size is obtained and then to trim. All the creases and folds should be carefully smoothed out, but undue stretching avoided. When templates are used it is difficult to get the cloth at the correct tension and to cut it without dragging.
4. Weigh the warp, weft and cloth accurately.
5. "Wet out" the warp and cloth in hot water, and, if salts such as magnesium chloride, etc., are present, give several rinsings, as such salts destroy the activity of the malt extract in which the yarn and cloth are to be placed.
6. Steep in a solution of malt extract and water at 60° C. for at least 15 minutes, or in a solution of "Rapidase" at about 90° to 95° C. for a few minutes. No particular strength of malt solution need be specified, but it should be fairly strong; 5 to 6 grms., stirred into about 200 c.c. of water, will give a strong enough solution. There is no need to keep the temperature constant; the yarn and cloth can remain in the cooling solution. All the starches are quickly converted into dextrin and sugars and can be removed with comparative ease.
7. Wash with warm water and "work" well.
8. Boil in soap solution for 3 to 5 minutes. (This consists of about 1 to 1.5 grm. of Hudson's soap dissolved in 200 c.c. of water.) This quickly

gets rid of the converted starch and also helps to remove any China clay. The duration of boiling should be as short as possible, to keep the "scouring loss" down to the minimum.

9. Wash thoroughly, working and squeezing well. By squeezing out the water and letting it drop into a beaker standing on a dark bench it is quite easy to see when all the china clay has been removed. It is always advisable to boil the cloth and yarn separately; some threads come away from the cloth and very easily get entangled with the warp threads if not kept separate. All loose threads must be carefully collected and kept with the cloth.

10. Dry in the water oven; the time taken will, of course, vary according to the weight of the cloth, but, roughly speaking, from 30 to 45 minutes is long enough. If all possible speed is required, soak the yarn and cloth first in alcohol and then in ether and dry in the oven for about 10 minutes.

11. Condition in a cool room for at least  $1\frac{1}{2}$  hours—longer if possible.

12. Again weigh. The losses in weight are calculated as the percentage of size on cloth and warp. It is necessary to convert grms. to grains, if the warp, weft and cloth have been weighed in grms.

*To find the count of a yarn the calculation is:*

$$\frac{\text{Yards taken} \times 7000}{\text{Grains found} \times 840} \quad \text{or} \quad \frac{\text{Yards} \times 25}{\text{Gr.} \times 3} = \text{Count.}$$

(Grms.  $\times 15.432$ )

*To find the weight of the cloth the calculation is:*

$$\left( \frac{\text{Weight in grs. of test piece}}{(\text{grms.} \times 15.432)} \right) \times \left( \frac{\text{reqd. width} \times 36 \times \text{reqd. length}}{7000 \text{ (grs. in 1 lb.)}} \right) = \text{Weight in lbs.}$$

Size of test piece in inches

Say,  $4'' \times 4'' = 1.376$  grm.; find weight for  $36'' \times 100$  yards;

$$\frac{1.376 \times 15.432 \times 36 \times 36 \times 100}{4 \times 4 \times 7000} = 24.57 \text{ lbs.}$$

From the particulars obtained the weights of warp and weft required for the cloth are calculated. These, when added together, should give approximately the same weight as the "after boiling" weight already found, and the addition of the percentage of size found on the warp should give a weight approximately equal to the "before boiling" weight.

The calculated results should come within about  $\frac{1}{2}$  lb. of the weight found by analysis. The full analysis can be completed in just over 3 hours. Three or four patterns can be started in the morning, and are easily ready for the final weighings early in the afternoon.

**NOTES ON THE METHOD.**—Taking it for granted that the analysis has to be done quickly, it is impossible to allow any great length of time for conditioning,

re-conditioning or drying; on the other hand, sufficient time must be allowed after the boiling out and drying for the yarn and cloth to attain to something like a normal condition.

In most contracts where a definite weight is specified, that weight has to be "not less than  $x$  pounds," and care is usually taken that the cloth is woven with yarns that will give a weight slightly over that specified, so that, even under the driest conditions, the cloth will not be too light.

As the result of many tests it has been found that, after the yarns and cloth have been boiled out and dried, conditioning for  $1\frac{1}{2}$  hours will allow the cotton to regain sufficient moisture for its condition to be considered fairly normal. For instance, a yarn may show 18.75<sup>a</sup> after  $1\frac{1}{2}$  hours' conditioning; if left overnight, in the same atmosphere, and re-weighed, it may then show 18.3<sup>a</sup>. This slight difference is immaterial to the manufacturer, as, whatever result is given to him, he will still base his price on 18<sup>a</sup> yarn.

"*Boiling out.*"—The treatment with malt diastase, or other solution containing diastase, followed by a few minutes' boiling with soap solution, does not damage the yarn or cloth, and the loss in weight of the cotton, apart from the removal of sizing materials, is only very slight. Blank tests on yarns free from size showed losses ranging from 2.5 to 4.0 per cent., according to the quality of cotton from which the yarns were manufactured. (Cf. Fargher, Hart and Probert, *J. Text. Inst.*, Jan., 1927).

As the sized yarn will always lose some of the size during weaving, the scouring loss due to boiling out should not be deducted from the percentage of size found by analysis, but should be taken to balance roughly the amount lost during the weaving.

At the same time an allowance should always be made for the fact that the count of yarn and weight of cloth found after removal of size are from 2 to 3 per cent. on the light side.

## A New Development in Filter Papers.

By E. J. GUILD.

(Demonstrated at the Meeting, May 6, 1931.)

My justification for bringing this filter paper (Whatman No. 54) to the notice of the meeting is that I believe it to be unique in its properties; these, briefly stated, are as follows:

(1) Extraordinary strength in water, *i.e.* when wet; its strength when wet transcends that of any usual filter paper.

(2) Great resistance to alkaline solutions, such as caustic soda, ammonia, etc.

(3) **Rapidity of filtration.** It will deal with large quantities of liquid where the matter in suspension is coarse or gelatinous. It will not retain fine particles; unfortunately, no one has yet succeeded in combining retentiveness and rapidity of filtration in one paper, but I may add that papers which are retentive and possess this outstanding wet strength are also available (*viz.* No. 52, which is medium retentive, and No. 50, which is highly retentive).

(4) **High degree of purity.** The alpha-cellulose is 99 per cent. or over, and the copper number is as low as 0.12 (Schwalbe-Braidy method).

(5) **Low ash content.** The percentage of ash is about 0.04, and this is mainly of an inert nature, so that the paper is suitable for all but the most highly delicate analytical work.

It will be appreciated that this paper should be very valuable for filtrations on a large scale, as it will withstand the pressure of large volumes of water or other liquid without risk of bursting.

#### DISCUSSION.

In reply to questions by the President and various members, Mr. Guild said that the paper was not cheap; it cost the same as acid-washed paper, but was more effective. He had not tried the paper for filter-press work, but saw no reason why it should not serve the same purpose as cloth. He had filtered caustic soda solutions up to 50 per cent. in strength without the paper showing signs of attack.

### Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

#### THE TITRATION OF QUININE IN ULTRA-VIOLET LIGHT.

It is well known that if a minute amount of quinine is added to sulphuric acid, the acid may be titrated with alkali, the end-point being reached when the fluorescence due to the quinine sulphate is destroyed. If the titration is carried out in a dark room provided with a source of ultra-violet light, the change is very striking, and gives an accurate end-point in solutions of 0.0001 *N* or less strength.

So far as I am aware, this method has not hitherto been applied to the determination of quinine itself. Anhydrous quinine was prepared by extraction of an ammoniacal solution of quinine sulphate with chloroform, the extract was evaporated, and the residue dried at 105° C. until constant in weight. A weighed portion (300 mgrms.) was then dissolved in 200 c.c. (*i.e.* an excess) of 0.01 *N* sulphuric acid, 20 c.c. were pipetted into a beaker, and titrated with 0.01 *N* sodium hydroxide

solution under a vertical beam of ultra-violet light. The end-point is reached when the excess of sulphuric acid over that required for the formation of quinine sulphate,  $(C_{20}H_{24}N_2O_4)_2 \cdot H_2SO_4$ , is neutralised. Further addition of alkali liberates free quinine, the presence of which produces a marked decrease in fluorescence of the quinine sulphate, which is easily seen by the naked eye in ultra-violet light. The end-point is not so sharp as in the direct acid-alkali titration with quinine as indicator, since it involves only a decrease in the fluorescence and not its complete disappearance; but, with practice, an accuracy of at least 0.1 c.c. of 0.01 N alkali is attainable, especially if comparison is made with two similar solutions adjusted previously one to each side of the end-point.

It is an advantage to standardise the alkali against the acid to the same end-point by addition of a minute amount of quinine to the latter, and titration with the former until the fluorescence, viewed in ultra-violet light, disappears. This end-point is, of course, extremely sharp, and the amount of quinine required is so small as not to affect the accuracy of the titration by using up the acid to form sulphate.

It is advisable to carry out the titration in a beaker placed on a white tile underneath the source of filtered ultra-violet light, since, if a conical flask or a horizontal beam of light is used, any fluorescence from the glass itself may obscure the end-point.

The result is obtained from the usual factor, *i.e.* 10 c.c. of 0.01 N sulphuric acid  $\equiv$  0.0324 grm. of quinine. Of the 20 c.c. of solution pipetted out for the titration, about 10 c.c. are used up by the quinine under the above conditions.

Comparative tests of the method against ordinary back-titration with brom-cresol purple as indicator showed that the same degree of accuracy is obtainable. Additional advantages are:—(1) Non-fluorescent substances (including slight colour and turbidity) which may obscure the indicator end-point do not interfere. (2) Errors inherent in indicator methods (*e.g.* due to the acidity or alkalinity of the indicator solution added, or to salt errors, etc.) are eliminated, since the substance titrated serves as indicator. (3) The purple colour of brom-cresol purple in solutions of about pH 6 is unstable on account of absorption of carbon dioxide from the air, and fading at the end-point often occurs if the solution is vigorously stirred; the method described has no such disadvantage.

Disadvantages are:—(1) The inconvenience of working in a dark room illuminated only by ultra-violet light, and especially of reading the burette near the end-point. I have found that if a small area of the bench is enclosed with dark curtains the determination may be made in the lighted laboratory, the burette being arranged so that the upper portion projects through the top for reading purposes. (2) As already stated, the end-point is not sharp to the unpractised eye, but this is to some extent offset by the fading of brom-cresol purple, and a number of titrations made in duplicate by means of the two methods gave identical results.

Extensions of the method suggest themselves and are being examined at the Hackney Technical Institute, London, E.8, where the above observations were made.

JULIUS GRANT.

### POISONING BY AMMONIA.

On December 13th, 1929, a female (Tamil) coolie, aged 32 years, employed on a rubber estate, was admitted to hospital, suffering from shock, having attempted to commit suicide by taking some poison. She is said to have taken curry and

rice at 7.0 p.m. and to have swallowed the poison half an hour afterwards. She died at 1.45 a.m. on December 14th. There was no vomiting or purging. The *post-mortem* examination was carried out at 3.15 p.m. on December 15th, about 36 hours after death, by which time decomposition was somewhat advanced. There were no external signs of injury. The outer wall of the stomach, left lobe of the liver, and several feet of adjacent small intestine were congested and discoloured dark red. There was congestion and marked reddening of the mucous membrane of the mouth and gullet. The other internal organs were normal, including the lungs. The stomach, on opening, smelt strongly of ammonia; it was found to contain a quantity of partly digested rice, reddened with blood, together with shreds of detached mucous membrane from the stomach lining. The stomach lining itself was highly inflamed and of a deep red colour, and the mucous membrane was severely corroded and partly destroyed.

The stomach and contents were examined separately. After mincing, half was diluted with water and distilled with steam until the distillate no longer reacted alkaline to litmus.

An aliquot portion of the distillate, which smelt strongly of ammonia, was titrated with  $N/10$  sulphuric acid, with methyl orange as indicator. By this method 17.5 grms. of ammonia were found. After communication with the police, it was found unnecessary to examine the remainder of the viscera. The quantity of ammonia found indicates that at least 25 c.c. of strong aqueous ammonia had been taken. Strong ammonia is readily obtainable on some rubber estates, where it is used as an anti-coagulant and preservative of latex.

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## Notes from the Reports of Public Analysts.

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF BIRMINGHAM.

#### REPORT OF THE CITY ANALYST FOR THE FIRST QUARTER, 1931.

OF the 1274 samples examined under the Food and Drugs Act, 134 were formal and 1140 informal. Seventy-nine samples were incorrect or adulterated.

**CRYSTAL MINTS.**—A sample contained 850 parts per million of sulphur dioxide. From the composition it was calculated that not more than 150 parts should be present. The vendor was cautioned, and intimated that immediate steps were taken to conform with the Preservatives Regulations.

**AMMONIATED QUININE TABLETS.**—Ten samples of ammoniated quinine tablets were taken. These are manufactured as a substitute for the liquid tincture and are usually made by mixing the requisite weights of quinine sulphate and ammonium carbonate with sufficient binding material to form a tablet. Of 9



samples, made with quinine sulphate and ammonium carbonate, all of which were probably originally of correct composition, 6 had lost practically all the ammonia, and 2 had average losses of about 80 per cent. The remaining sample was made with ammonium chloride instead of ammonium carbonate, the proportion of the two substances being declared on the label. These proportions were found to be as stated, ammonium chloride not being volatile.

In one case the wholesale dealer, at the request of the manufacturers, agreed to attach a label to the bottle worded as follows: "These tablets contain the same percentage of quinine as in a dose of the tincture, but no guarantee can be given in respect to the quantity of ammonia owing to its volatile nature."

H. H. BAGNALL.

## GIBRALTAR.

### REPORT OF THE CITY ANALYST FOR THE YEAR 1930.

THE total number of specimens and samples examined was 4776. Of the 124 foods and drugs examined, 18 were below the standard. A careful watch was kept on the purity of the drinking waters of Gibraltar, 354 samples being examined bacteriologically.

**GOATS' MILK.**—The statutory limit for milk-fat, fixed by the Public Health Ordinance, is 3·5 per cent. The average composition of the 47 samples examined was: Fat, 4·18; solids-not-fat, 8·89 per cent. Ten were deficient in milk-fat as the result of skimming by the vendor. This is an increase of 11 per cent. over last year's figures. Vendors appear to be at liberty to take off the fat which rises quickly to the surface after boiling, and offer the skimmed or partly skimmed milk to the public at the same cost as whole milk. The law is evaded by declaring to the Sanitary Inspectors at the time of purchase that the milk is skimmed. No such statement appears to be made to the general public, who unwittingly are deprived of some of the milk-fat. It is satisfactory to report that no sample of goats' milk was offered to the public unboiled. A bacteriological examination was made to ascertain whether a milk vendor was effectively sterilising goats' milk sold in bottles as "sterilised." The following results were obtained:

	Sample before sterilising.	Sample sold as "sterilised."	Sample after correctly sterilising.
<i>B. coli</i> .. .. .	1,000,000 per c.c.	not in 10 c.c.	not in 10 c.c.
Streptococci .. .. .	1,000 per c.c.	not in 10 c.c.	not in 10 c.c.
"Enteritidis" change ..	in 30 c.c.	not in 30 c.c.	not in 30 c.c.
Organisms at 37° C. per c.c. ..	42 millions	520	12
Organisms at 22° C. per c.c. ..	75 millions	152	13

The serological agglutination test was carried out on the 162 goats living on the "Rock." All gave negative results for undulant fever.

**METALLIC CONTAMINATION OF AERATED WATERS.**—Continued supervision of the manufacture of aerated waters in Gibraltar revealed the presence of lead in harmful quantities in the soda water of one factory. Solder containing lead was found in the carbonator and has been removed.

**TESTING OF SHIPS: Inflammable and Poisonous Vapours.**—The City Analyst is entrusted with the testing of compartments of oil-carrying ships and others for dangerous gases. Such work was first undertaken for Naval Authorities in 1928,

but has since been extended to local shipping agents. Gas-free certificates are necessary before ships of this class enter dry dock for repairs.

Two ships were examined:—(1) A tanker of nearly 6,000 tons, which was certified gas free. (2) A cargo ship on which a fire had occurred in the mixed cargo of foodstuffs, etc. The ship had been flooded. Inflammable and poisonous gases were present beneath the decomposing materials, and the agents were warned of the risks to the men when entering the holds to unload.

A. G. HOLBOROW.

## CITY AND COUNTY OF KINGSTON-UPON-HULL.

### ANNUAL REPORT OF THE PUBLIC ANALYST AND BACTERIOLOGIST FOR THE YEAR 1930.

DURING the year 1930 the number of samples of all kinds examined was 6902, of which 1620 were samples for chemical analysis, and 5282 were specimens for bacteriological examination. Of the 1247 samples examined under the Food and Drugs Act, 20 were suspicious and 48 adulterated.

"DIRT" IN MILK.—Thirteen of the 652 samples of milk received from the Sampling Officers under the Adulteration Acts were found to contain unwarranted amounts of extraneous matters (dirt), a percentage of 2·0. This figure is slightly lower than that recorded last year (2·3 per cent.). Three of these thirteen samples were the unsatisfactory ones of a total of 12 milks taken specially for examination for dirt; these were three-pint samples, allowing after division one pint for the determination of extraneous matters. These three samples were found to contain 5·5 parts of dirt (sand and dung), 4·0 parts (partly dung), and 2·0 parts (sand, etc.) per 100,000.

CURDS.—The sale of wet milk-curd for making curd cheese-cakes is common in the City, and an examination of twelve samples was made during the year. They contained from 74 to 81 per cent. of water, and all were free from foreign additions, including alum. It has frequently been stated that alum is used in curdling the milk for curd-making, but if used, it is carefully washed out. It is very necessary that such washing of the curds with water should be thoroughly done, as the presence of alum in foods must be regarded as an adulteration. It would be a better practice to use a rennet preparation rather than alum for making these curds. There is no doubt that by carefully draining the washed curds, the amount of extraneous water can be reduced, and perhaps 75 per cent. might be regarded as a maximum. On such a basis, about half the samples examined contained water in slight excess.

DRIPPING.—Twelve out of fifteen samples were of satisfactory composition, being sound edible beef-fats free from water and other extraneous substances. One of the three unsatisfactory products was unsound, since it contained 7·2 per cent. of free fatty acids and gave definite reactions showing a rancid condition. The vendor was cautioned. Two other samples (informal and formal samples from the same vendor) were returned as adulterated, and proceedings instituted, since the "dripping" supplied contained 15·3 and 22·8 per cent. of water, with tissue, salt and other extraneous ingredients. These samples were obviously pork dripping with much of the gelatinous gravy mixed with the fat, and it was contended by the defence that the ordinary standards could not apply to a product which was sold at a good price because of its superior flavour, due to the presence of the meat extractives. The case was dismissed, but the position, as left by the decision,

was not quite satisfactory, since it cannot be denied that "the fat which drips from roasting meat" is a reasonable definition of dripping. However, as a result of discussions with representatives of the trade concerned, the vendors of such products have agreed to label them as "pork dripping with gravy."

**SHREDDED SUET.**—Forty-three samples examined in the City Laboratories during the last six years showed an average addition of rice or other flour of 11.5 per cent., and more than one-third of these samples contained not more than 12 per cent. It is clear that 10 to 12 per cent. of flour is a reasonable amount if the addition is declared on the label, and that an allowance of another three per cent. (15 per cent. addition in all) should cover all reasonable variations.

**SPONGE CAKE MIXTURES.**—Six samples were composed of cereal or other flours (wheat, maize, potato and mixtures of these) and sugar, together with small amounts of baking-powder ingredients. Four of these mixtures were coloured with a permissible coal-tar dye (yellow or pink). Some little time may be saved by using these products in cake-making, but they are costly in proportion to the amount of nutriment purchased. The ingredients cost (retail prices) approximately, on the average, perhaps 2d. per lb., whilst the mixtures were sold at prices ranging from 9d. to 10½d. per lb.

**POTTED MEATS.**—All the twenty-one samples of loose potted meats, sold with a fatty covering, were sound products as regards freedom from souring, and from objectionable ingredients other than farinaceous material and excess of water. Only one sample contained a slight excess over the proportion of water regarded as reasonable; this meat contained 72.8 per cent. of water as compared with 51 to 70 per cent. in the remainder. The last-mentioned figure should not be exceeded. Eight of the samples contained an added starchy constituent, frequently amounting to about 10 per cent. of the foodstuff, but the remaining 13 samples contained no such addition. Potted meat should be cooked meat, finely minced, and containing nothing else but the natural extractives (gravy) of the meat. The best makers conform to the standards here laid down. Other products containing extraneous additions should be sold as meat pastes. It is time that these standards were enforced in the City, and that those products not conforming to such standards were considered adulterated within the meaning of the Food and Drugs (Adulteration) Act, 1928.

**GLASS PARTICLES IN FOODS.**—No sample of glass-packed food has shown any appreciable traces of glass particles. Since the year 1924, when the matter was first mentioned in these Reports, there has been a progressive improvement in the quality of the glass containers used for packing foodstuffs, and there is now little likelihood of this form of contamination except as an accidental occurrence.

**BACTERIA IN COMMERCIAL MILK.**—Nine samples of ordinary *commercial milk*, submitted by the Medical Officer of Health, were examined bacteriologically. Judged by the standards for Grade "A" milk, two samples were somewhat unsatisfactory, showing under 200,000 organisms per c.c., but having *Bacillus coli* in 1/100th c.c. of the milk.

**PETROL IN SEWAGE.**—In connection with an investigation into the cause of a serious explosion in West Dock Street, undertaken in October at the request of the Deputy City Engineer, samples of the sewage and "air" in the main sewer of this street were collected for examination. The "air" contained a considerable quantity of vapour inflammable at the ordinary temperature, and the sewage was found to have, floating on its surface, a light oily layer with an odour of petrol, and which showed on distillation an initial boiling point of 56° C. The presence of liquid and vaporised light petroleum spirit in the sewer thus proved that the

explosion was caused by the ignition, in some way, of petrol which had obtained access to the sewer. The dangerous mixture of air and petrol-gas formed in the sewer would be at least one thousand times the volume of any petrol run into the sewer. The City Engineer caused a warning notice to be issued to all persons concerned.

**SUNLIGHT (ULTRA-VIOLET RAYS) OBSERVATIONS.**—These recordings have been continued throughout the year at the Central observation station. It has become increasingly evident that, whilst useful results can be obtained with the acetone and methylene blue solution, provided that due care is taken, the method involves several fallacies, and that, unless these are guarded against, the results may be much too high.

The table below gives the maximum and minimum daily averages for various towns:—

*Records of Ultra-Violet Light.*

Place.	Units of Fading. Daily average throughout the months mentioned.	
	Maximum.	Minimum.
Hull (Central) .. ..	5.5 (June)	0.08 (Dec.)
Cardiff (Central) .. ..	2.8 (June)	0.4 (Dec.)
Edinburgh .. ..	2.3 (Aug.)	0.2 (Feb.)
Lytham St. Anne's .. ..	3.2 (May)	0.3 (Dec.)
Lowestoft .. ..	9.9 (June)	0.9 (Dec.)
Prestatyn .. ..	6.1 (June; Aug.)	1.4 (Dec.)
Rochdale (8 months only)	1.1 (Aug.)	0.03 (Dec.)
Skegness .. ..	7.0 (Aug.)	0.9 (Feb.)
Stirling (Central) .. ..	3.3 (June)	0.2 (Jan.)
Walsall (7 months only)	3.2 (Aug.)	0.02 (Dec.)

A. R. TANKARD.

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### VALIDITY OF PATENT FOR TREATMENT OF CHEESE.

ON July 17th the Judicial Committee of the Privy Council (comprising the Master of the Rolls, Lords Tomlin, Russell of Killowen and Macmillan, and Sir L. Sanderson) gave judgment in the appeal by James Lewis Kraft, the Kraft Cheese Co. (Incorporated) and the Kraft Cheese Co. Proprietary, Ltd., in the matter of their patent (No. 1620 of 1916) in Australia, for a process of making cheese of the Cheddar type keep permanently. (A similar patent exists in Great Britain.) The respondents were Oliver Kenneth McAnulty, carrying on business as the Maxam Cheese Factory in Australia.

The Master of the Rolls (Lord Hanworth), in giving judgment, said that this was an appeal from a decision of the High Court of Australia reversing a decision of the Supreme Court of Queensland in favour of the appellants. It was alleged

by the appellants that their patent for treating cheese in such a way that it would keep indefinitely, and without spoiling, had been infringed by the respondents.

The respondents denied that they had infringed the patent, and claimed that it was invalid for reasons which included want of subject matter, want of utility, and further claimed that the patent specification was ambiguous and misleading. The Court of Queensland held that the patent was valid, but on appeal this decision was reversed by the High Court of Australia, by a majority of three judges to two, on the grounds:—(a) That the patent was invalid because the representation or promise of complete sterilisation at about 175° F. was not true; (b) that the specification was ambiguous and misleading and did not disclose, or prescribe, a method whereby the object aimed at could be achieved.

Special leave to appeal was given, and the hearing was on June 15th, 16th, 18th, and 19th. The Master of the Rolls pointed out that two views were presented upon the construction of the specification. The one that the main desideratum was complete sterilisation; the other dwelt upon the statement: "the invention consists in the process of rendering the cheese . . . permanently keeping."

The majority in the Court of Appeal held that the phrase "permanently keeping" was used to denote cheese that had been both completely sterilised and so placed as to maintain complete sterilisation. They were unable to cut down the meaning of sterilisation to the degree for which the appellants contended. Then came the question, was the cheese, in spite of that, a permanently-keeping cheese?

The Judicial Committee held that they could not accept the view of the Queensland Court and of the two judges of the High Court of Australia, that the evidence proved that cheese made by the Kraft process was "permanently keeping."

Their Lordships found themselves in agreement . . . that the evidence suggested to "say the least, a very modified permanence." They also pointed out that in both claims there was some insufficiency in the description of the process to be followed. The principle in patent law was clear, that a patentee must define the nature of the invention and disclose a process which would produce the result aimed at.

Their Lordships would humbly advise His Majesty that the judgment of the High Court be affirmed and the appeal dismissed with costs to be paid by the appellants.

#### ARTIFICIAL PRODUCT SOLD AS GRAPE VINEGAR.

At Liskeard Police Court, on September 2nd, a manufacturer, trading as the Grape Vinegar Co., was summoned for selling pure grape vinegar which was not of the nature, substance and quality demanded by the Cornwall County Council's inspector; there was a further summons for affixing a false label. Inspector James said that he took a sample in course of delivery from one of the defendant's casks, which was labelled: "Pure Grape Vinegar." The defendant's invoice bore a picture of a bunch of grapes, and this liquid was supplied by a retailer (who was also summoned), when asked for grape vinegar. The Public Analyst, Dr. H. E. Cox, said that, on analysis, the sample was found to consist of diluted and coloured acetic acid, and was artificial vinegar; it contained no phosphate, tartrate or inositol, which would be found in small quantities in the product of the grape. So far as he knew, there was no grape vinegar manufactured in England, though it was known on the Continent and valued for its flavour.

The defendant urged that by calling his product "Grape Vinegar" he had only given it a name and did not intend to imply that it was derived from the grape. The grape was a symbol of purity and excellence; if he had called it "Pyramid Vinegar," would the Public Analyst have expected to find traces of the pyramids in it?

The Bench imposed a fine of 40 shillings, with two guineas costs.

The retailer was fined £1 for adding 20 per cent. of water to the vinegar.

### EGG POWDER—GUARANTEED TO CONTAIN EGG.

At Saltash Police Court, on August 25th, a retail grocer was summoned for a breach of Section 2 of the Sale of Food and Drugs Act, in that he sold to the Cornwall County Council's Inspector two tins of egg powder which were not of the nature, substance and quality demanded. The powder in question was labelled: "—'s Real Egg Powder, Double Strength, Guaranteed to contain the Real Yolks of Eggs." It also bore a facsimile of a certificate of a Fellow of the Institute of Chemistry, dated March, 1894, which certified "that an essential ingredient of its composition is sound yolk of egg." The certificate of the Public Analyst, Dr. H. E. Cox, showed that the powder was composed of tartaric acid, acid sodium pyrophosphate, bicarbonate of soda, rice starch and a yellow dye, and that it contained no egg. He said that a powder described as real egg powder containing eggs, as this one was described, ought to contain a substantial proportion of egg; the sample in question was really an egg substitute; the expression "double strength" was meaningless in application to this material.

Mr. C. Knight, for the County Council, explained that the retailer had not pleaded a warranty, and the wholesaler or manufacturer was not in the county, so he was obliged to proceed against the retailer. Mr. Wolferstan, for the defendant, agreed that the composition given by the County Analyst was correct, but urged that the powder had been manufactured for about 40 years from the same formula, although he did not know how it came about that acid pyrophosphate was present, a product which was not in use 40 years ago. It had been awarded various gold medals. A representative of the manufacturers said that the analyst's certificate appearing on the label had since been withdrawn.

The Bench intimated that the offence had been proved, but as they sympathised with the retailer, who had no intention of committing an offence, they imposed a fine of only ten shillings.

## The National Physical Laboratory.

### REPORT FOR THE YEAR 1930.\*

THE volume includes the Report of the Executive Committee; a Comparison of Tests for the last three years; a list of Official and Unofficial Published Papers, and the detailed reports of the Physics, Electricity, Metrology, Engineering, Aerodynamics and Metallurgy Departments, and also that of the William Froude

\* Department of Scientific and Industrial Research. Obtainable at Adastral House, Kingsway, W.C.2. Price 12s. 6d. net.

National Tank. From the mass of material a few investigations may be specially quoted.

**PHYSICS: Maintenance of Standards. Optical Pyrometer Scale.**—The work on melting points at high temperatures has been continued by obtaining a black-body radiator, consisting of a tube immersed in an ingot of the metal which can be held a sufficient time at the melting point to allow observations by the pyrometer to be taken. The small ingots are heated either by indirect induction by means of a heating unit consisting of molybdenum foil wrapped round a thoria tube fixed in a silica tube, or by direct induction by heating by a coil providing the maximum ampère turns per unit length, fitted in an alundum tube into which is inserted a cylindrical thoria crucible containing the ingot (0.6 cm. in diameter and 1.8 cm. long), threaded with a thin-walled alumina tube. The black-body radiator consists of a small fragment of alumina nearly of the same cross section as the tube at its centre. Although reliable determinations were obtained with these small ingots of platinum, the work is to be repeated with a thoria crucible and radiator presented by the Bureau of Standards, permitting the use of ingots, 1.6 cm. in diameter by 3.5 cm. long, and, if successful, the radiator might at the same time be used as a standard of photometric intensity.

**GOVERNMENT RESEARCH, ETC.**—Work has been carried out for the Food Investigation Board on heat transmission to cold pipes, and a single pipe is being studied in air of varying humidity and with speeds up to 30 ft. per second. The Determination of Water in Fogs (work carried out for the Atmospheric Pollution Research Committee) having been impeded by lack of natural fog, artificial continuously dense fogs have been used. Owing to difficulties experienced with Kohler and Owen's apparatus, a gravimetric method has been tried which measures the water as droplets by aspirating the air at about 60 cb. ft. per hour through U-tubes of 2 in. bore, containing wetted glass wool, and measuring the volume by a dry gas meter. A dew-point method has also been used in which the foggy air is heated to evaporate the droplets, and the dew-point is determined on the fog-free air.

Work for the Building Research Board was done on transmission tests for various materials, including wall sections of closely thatched straw, cement faced one side and plastered the other, and one of reeds instead of straw.

**Action of Heat on Iron Oxides.**—In collaboration with the Research Association of British Paint, Colour and Varnish Manufacturers, the effect of heat on iron oxides was made the subject of X-ray examination. Freshly prepared hydrated iron oxide ( $\text{Fe}_2\text{O}_3 \cdot 2\text{H}_2\text{O}$ ) was amorphous;  $\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$ , prepared commercially, gave a diffraction pattern corresponding with a structure not yet worked out, and, on heating, the water was not wholly removed under  $300^\circ\text{C}$ ., after which the structure of the oxide ( $\text{Fe}_2\text{O}_3$ ) was a cubic one, resembling that of  $\text{Fe}_3\text{O}_4$ , but with a slightly different intensity distribution. Further heat treatment caused a change to trigonal  $\text{Fe}_2\text{O}_3$ , beginning at  $500^\circ\text{C}$ . and complete at  $600^\circ\text{C}$ .

**COLOUR MEASUREMENT AND STANDARDISATION.**—The three-colour mixture curves, based on the spectrum observations of seven observers, are so far confirmed in their accuracy that the data have been put into a form for general use, and specific proposals based on these and other published data are being put forward for the definition of the "normal" eye. The standardisation of the 57 colours, scheduled as standard for ready-mixed paints by the British Engineering Standards Association, has been completed and results incorporated in the British Standards Schedule of Colours for Ready-mixed Paints. The Pharmacopoeia Commission

was assisted in its colour specification for the antimony trichloride test for cod-liver oil, and the Fuel Research Station in specifying a set of colour standards for testing coal ash.

**ELECTRICITY DEPARTMENT: *Photometry Division.***—The investigations relating to heterochromatic measurements have been continued and the visual spectrophotometer has been described (*J. Scientific Instruments*, 1930, 7, 306). It is sufficient to calibrate the standard lamp at one standard wave-length, and the calibration for other wave-lengths is deduced from Wien's law. The transmission of the Ives yellow and blue solutions for determination of the Y/B ratio for different observers has been measured, and records made on glasses for photometric determinations, so that a study of flicker photometry for large colour differences may be made. The spectrophotometer is being used for measuring the effective wave-lengths of coloured glass, which can be found from two measurements at different colours. A change of 20° C. in room temperature will lead to an error of 4° C. in the deduced melting point of platinum, owing to the influence of temperature on the red glasses of optical pyrometers.

**PHYSICAL INSTRUMENTS.**—Work on the new standard barometer (ANALYST, 1930, 55, 512) has been continued, and comparisons with other standards are shortly to begin. Relative weights of the Laboratory standards of Mass have been re-determined and comparisons made with the standards of the Standards Department of the Board of Trade. The ampère balance has been reconditioned, and can now be used for accurate determinations of the moment of inertia of the suspended system.

**VOLUMETRIC GLASSWARE AND HYDROMETERS.**—The drafting of specifications for the adoption, throughout the Empire, of uniform specifications and methods of testing glassware used in the examination of milk and milk products is in hand. Methods of testing centrifuge tubes for the determination of dirt in milk are being investigated, and the leakage test for burette stopcocks is under review. In collaboration with the Standardisation of Tar Products Tests Committee, specifications for glassware and hydrometers to be used in testing tar and its products have been prepared, and a series of experiments made to enable suitable hydrometers to be specified for tar and tar distillates.

**ENGINEERING DEPARTMENT: *Lubrication.***—The Investigation for the Lubrication Research Department Committee into the characteristics of boundary lubrication for surfaces under relative reciprocating motion has been continued, and a machine constructed in which the frequency of oscillation has been reduced to 10 per minute, in order to reduce the inertia of the moving parts of the mechanism.

The work on "doped" lubricating oils for the Aeronautical Research Committee has shown that addition of lead ethyl and such substances to lubricating oils reduces the coefficient of friction and raises the seizing temperature, but that the opposite occurs with castor oil. Three samples of oil from an asphaltic base showed a marked reduction of the minimum friction to be produced by oxidation or by addition of lead ethyl, but, if they had been previously oxidised, no further reduction occurred.

**METALLURGY DEPARTMENT.**—The X-ray spectrometer has been particularly applied to the study of the aluminium-copper system, and two new lattice structures have been discovered, probably corresponding with definite phases in the region between 20 and 30 per cent. of aluminium. Variations detected in the lattice parameter of iron-chromium alloys are being further investigated, and the work on gold-copper alloys of composition approaching that of the compound AuCu has shown that the depression of the transformation temperature is due to slight



absorption of oxygen. Experiments in the determination of elastic constants of inter-metallic compounds by production of acoustical vibrations in small specimens are in progress.

Work on the production of single crystals of pure metals has been continued, together with further work on the preparation of pure metals, particularly iron, with a view to preparing larger quantities for corrosion research work. The surface tension of mercury has been measured between  $0^{\circ}\text{C.}$  and  $-37^{\circ}\text{C.}$ , and that of tin-lead alloys down to  $240^{\circ}\text{C.}$  Investigation on aluminium alloys have shown that the dimensional changes occurring on machining are greatly reduced by quenching in boiling water or oil, and that there is no change in mechanical properties.

The work on the removal of dissolved gases from molten aluminium alloys. The good results obtained by passing volatile chlorides, particularly titanium tetrachloride, have been published (*J. Inst. Metals*, 1930, **44**, 305), and the laboratory results have been confirmed on a large industrial scale, so that ingots and castings have been obtained substantially free from gas cavities.

Welded joints in aluminium alloys are under investigation, as are also the mechanical properties and resistance to corrosion of magnesium alloys. A paper dealing with the results of work on nickel-chromium and iron-nickel-chromium alloys has been published (*J. Iron and Steel Inst.*, 1930, **121**, 225), and it has further been found that the greatest resistance to prolonged stress at high temperatures is exhibited by an alloy containing: Silicon, 1.0; carbon, 1.5; tungsten, 4; nickel, 30; chromium, 30; and iron, 33.5 per cent.

The important study of the resistance of various steels to oxidation by flue gases has been begun. An investigation into "creep" is in progress, and work on gases in steels and on the cracking of boiler plates is also being carried out for the Metallurgy Research Board. Continuous immersion in tap-water has been found to reduce the life of boiler plates by 30 to 40 per cent.

D. G. H.

## Government of Madras.

### ANNUAL REPORT OF THE CHEMICAL EXAMINER FOR THE YEAR 1930.

In his Annual Report to the Local Self-Governing Department of Madras, Lieut.-Col. C. Newcomb, M.D., F.I.C., mentions that in the course of 1931 the Chemical Examiner was to be relieved of his professorial work at the Medical College, leaving him free to attend to his medico-legal work, and to undertake research on the identification of certain poisons which are not very uncommon in India, but are almost unknown in Europe.

The total number of articles examined during the year showed a slight reduction on 1929 (5406, as compared with 5844), but the human poisoning and the stain cases showed an increase, and there are no indications that this increase will not continue in future.

**HUMAN POISONING CASES.**—The number of cases investigated was 271, in which 330 persons were affected and 207 died. In 133 cases poison was found, mercury being identified in 21, arsenic in 10, atropine (or datura) in 18, and opium

in 16 cases. Among the less common poisons detected were oduvan (2 cases) and madar (1 case). The tests found the most useful for the detection of these poisons were as follows:—

**ODUVAN LEAVES.**—The acid ethereal extract gives a green colour with strong hydrochloric acid and kills a frog when injected under its skin. The reaction can often be obtained from the stomach contents in cases of poisoning.

**MADAR.**—(1) The juice itself or the stomach contents, freed from the alcohol in which they are preserved, give a green colour with strong nitric acid.

(2) On boiling the juice with alcohol under a reflux condenser an extract is obtained with a peculiar and rather characteristic smell. With stomach contents the difficulty in applying this test is that the offensive smell of the decomposing organic matter frequently obscures the rather pleasant odour of the madar, but sometimes the odour is recognisable even in the stomach contents.

(3) We have not had much success with the crystallisation test. In this the above alcoholic extract is allowed to evaporate in a glass dish, when crystals, resembling cauliflower, are stated to form. In our experience, the crystals are not at all characteristic in form.

(4) Should a considerable quantity of madar juice be available the resin can be extracted and purified, and its properties compared with that obtained from known madar juice, but in poisoning cases there is rarely enough of the poisonous substance available for this procedure.

It is not, of course, possible with either oduvan or madar poisoning to be certain from these tests of the exact nature of the poison, but the tests indicate the probable presence of the poison. This, together with the medical evidence of the symptoms, is often sufficient to establish with a fair degree of certainty the poison used.

**ANIMAL POISONING CASES.**—Thirty-three cases with 122 articles were investigated. In 17 of the cases poison was detected. Arsenic was found in 8 cases, and yellow oleander (or its active principle) in 7. In 2 of the cases an unidentified poison was found.

**STAIN CASES.**—During the year the number of stain cases investigated was 749 with 3217 articles. The number again exceeded that of the previous year, and was the largest on record. The Imperial Serologist examined 2296 specimens of bloodstains, of which 2182 were human blood.

**DETECTION OF SEMINAL STAINS BY MEANS OF ULTRA-VIOLET LIGHT.**—The use of an ultra-violet lamp, with a shade and a glass screen cutting off nearly all the visible light, has been very useful in finding semen stains on white or light-coloured cloths. On black or dark-coloured cloths ultra-violet light is useless, and one has to decide which portions to cut out for examination by eye and feel in the usual way—a tedious business with a large cloth. A white cloth can be passed rapidly through a beam of ultra-violet light, and any seminal stains will at once shine with a bluish fluorescence. A great many other stains which are not seminal show up too, and it is surprising what a number of different stains will appear when viewed in ultra-violet light on a cloth which, by ordinary examination, is fairly clean. Ultra-violet light is no test for semen, except in the sense that it shows one to which portion of the cloth to confine one's attention. This, however, when dealing with a large cloth, is no small advantage.

**Florence's Test for Semen.**—The number of rape cases in which we can find no semen (and by no semen I mean not only no spermatozoa, but no stains which will give Florence's test), is always a little surprising. One expects to fail to find spermatozoa in many seminal stains, as spermatozoa are extremely fragile and

readily decomposed and have been shown to disappear quickly from stains known to be seminal, but if a seminal stain is dried and kept dry—as most of those we get are—one expects to get Florence's test from it. If one does not, it is very unlikely that the stain is really seminal. I think a large number of rape cases must be false cases, and I am supported in this opinion by the fact that in many of the alleged seminal stains we examine, we find that the stains are those of starch.

**EXAMINATION OF BOMBS.**—Thirty-five miscellaneous medico-legal cases, with 160 articles, were investigated. Most of these cases were bombs of the type common in these parts, *viz.* a round packet containing red sulphide of arsenic, potassium chlorate, and small stones or nails, all tightly packed together in cloth and bound with string or cotton. Such a bomb explodes on percussion, and though, as bombs go, it is not of a particularly dangerous type, a well-made one can do considerable damage and can easily kill a man if it strikes near a vital part. In some of the bombs sent to us the bomb-maker had been deceived, by a superficial resemblance, into using the wrong materials. In one case what should have been potassium chlorate was found on analysis to be tartaric acid, and in another the red sulphide of arsenic had been replaced by potassium dichromate—a red substance somewhat resembling it. Neither of these bombs would have exploded.

**DETECTION OF ERASURE BY MEANS OF ULTRA-VIOLET LIGHT.**—In one of these miscellaneous cases we were sent a bus licence for examination for alteration of the figures on it. It was suspected that in the figure " $1\frac{1}{4}$  tons" the " $\frac{1}{4}$ " had been erased and subsequently re-inserted. On examination in ultra-violet light this suspicion was confirmed, and the erasure and subsequent insertion of the figure " $\frac{1}{4}$ " could be distinctly seen.

## Ceylon.

### REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1930.

IN his report on the work of the Government Analyst's Department, Mr. C. T. Symons mentions that, in the absence of any alterations with regard to staff and accommodation, and in view of the necessity for retrenchment, it became necessary towards the end of the year to inform the departments concerned that the work of the Department would in future be limited to the scope laid down in general orders, wherein the work of the Government Analyst is defined as: (a) Analyses arising out of criminal investigations; (b) examinations and other duties under the Petroleum Ordinance; (c) analyses of water or milk sent by a Government Department; with a proviso that, should an analysis of any other kind be required by the Head of a Department he should apply to the Government Analyst, showing special cause for his application.

The work discontinued includes scientific investigation of crime (other than chemical analysis), examination of firearms, examination of stains, analysis of food and drugs, analysis of sewage effluents, etc.

The following notes are given on some of the remarkable cases dealt with during the year.

**POISONING CASES.**—Specimens were examined in 104 cases, and in 33 of them poison was identified. Ten involved arsenic (in one case with a barium salt, and in another as Paris green), 8 prussic acid (in one case with morphine), 4 mercury,

as metal or as salt, 2 phenols, and 1 each, *Gloria superba*, morphine, chenopodium oil, zinc chloride, a copper compound, and powdered glass. A case of some interest was one in which no prussic acid was found in the viscera of a man who had undoubtedly died from the ingestion of potassium cyanide. Traces of the poison were found in the tumbler he used and in other receptacles in his room.

**BULLETS AND FIREARMS CASES.**—Twenty-three cases were investigated, necessitating the examination of 108 articles.

*Imprint of Cloth on Bullet.*—In one case from Colombo a man who was wearing a coat and shirt was shot through the arm. The bullet went through his arm, but after that did not penetrate the shirt and coat a second time. The next day a revolver bullet was found in the rubbish heap behind an adjoining bungalow. It was suspected that the man had been shot, at fairly close range, by a man using a revolver and intending to shoot someone else who was standing near. The suspected person admitted that he had fired several shots that night from the window of his bungalow, with the intention, he said, of frightening away a riotous crowd who were damaging his house and trying to break in, but he denied having wounded or fired at any person. A visit to the scene soon showed that his story was false, so far as it concerned the assault on his house. The slight damage had been done with the object of simulating an assault on the house. In addition, the wounded man was conclusively proved to have been standing in a position which could not have been reached by shots fired from the window, and finally, by means of the comparison microscope and bullet comparator, it was found possible to demonstrate that the bullet picked up in the rubbish heap had been fired through the accused person's revolver, and that it had actually penetrated through a piece of cloth of exactly the same texture as that of the coat of the wounded man. This was possible from the impression of the cloth left on the end of the lead bullet when it hit the man's arm, and from the rifling marks on the bullet, and its irregular entry into the barrel.

In another case, the problem sent up was more in the nature of a jig-saw puzzle, since the productions consisted of two tubular pieces of metal, a chopper, and some fragments of wood and metal, and the Analyst was asked whether the fragments originally constituted the stock and barrel of a gun, and whether the chopper had been used to cut it up. After some trouble the pieces of wood were fitted together to form a passable gun stock, and by a piece of good fortune a flaw in the blade of the chopper was found to contain a fragment of the same wood as the stock, an unusual kind of wood to use for firewood.

**SPECTROSCOPIC IDENTIFICATION OF TELEPHONE WIRE.**—In the past many cases have been initiated in the courts, charging various persons with the theft of Government copper telephone and telegraph wires. Most of these cases have been dismissed, on account of the difficulty of proving the identity of the seized wire with Government samples, in view of the defence that the wire was quite legitimately purchased from local importers. We were consulted on this subject, and by means of the Féry quartz spectroscope we were able, in a recent case, to demonstrate quite conclusively that the seized sample contained traces of certain ingredients which were present in Government wire, but not in any other samples obtained locally. This evidence is obtained very rapidly by a photographic process, and can be demonstrated by prints, showing that the finding is not a matter of opinion, but one of fact.

**IDENTIFICATION OF CLOTH BY MEANS OF ULTRA-VIOLET LIGHT.**—An extraneous torn piece of common white cloth was found in a house where a burglary had been committed. Another piece of similar cloth was found in a suspected

person's house. Each piece showed the same material, the same state of dirt, a similar seam and fresh tearing, but this was not sufficient to establish identity of source where such commonly used cloth was concerned. Examination under Wood's light, however, showed up certain stains, invisible to the naked eye, which extended over the freshly torn edges, and formed continuous stains when these edges were placed together. The nature and conformation of these stains conclusively established the fact that the two pieces of cloth had originally formed one piece.

In future, such cases as this will not be dealt with in the Government Analyst's Department.

**PROOF OF FRAUD BY MEANS OF RECEIPT STAMPS.**—In one case it was possible to prove and demonstrate fraud clearly by means of the perforation on receipt stamps. Three promissory notes were produced in support of an insolvency case and purported to have been made at two different places and at intervals of time covering about a year. The signatures were genuine, but were all written with the same pen and ink, which had been specially prepared to produce an appearance of age. The pen was not a fountain pen. The pen points showed progressive deterioration throughout the three documents, but not in the proper order. But the most conclusive proof was from the stamps, which, by the irregular tearing of the perforations, were shown to have formed originally one block of three stamps. (Cf. Mitchell, *Documents and their Scientific Examination*, p. 178.)

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Method for the Determination of Small Quantities of Mixed Reducing Sugars and its Application to Determination of Products of Hydrolysis of Starch by Taka-Diastase.** E. M. Widdowson. (*Biochem. J.*, 1931, 25, 863-879.)—Preliminary investigations on the carbohydrates in the developing apple have shown that the concentration of sugars in the fruit is less than 1 per cent. during the first weeks of growth, and that the maximum starch concentration reached at any time is only about 2 per cent. Since starch appears to be most satisfactorily determined by measurement of the glucose and maltose produced by hydrolysis with taka-diastase, a method for the determination of small quantities of reducing sugars has been investigated. In order to determine two mixed reducing sugars it is necessary to employ two methods of determination, and to solve the pair of simultaneous equations obtained from the results of the determinations. Hagedorn and Jensen (*Biochem. Z.*, 1923, 135, 46) have used the method of oxidation by alkaline ferricyanide for the determination of amounts of glucose ranging from 0.02 to 0.36 mgrm. in 2 c.c. Hanes (*Biochem. J.*, 1929, 23, 99; *ANALYST*, 1929, 54, 349) has applied this method to the determination of quantities of glucose and maltose up to 3.8 mgrms. in 5 c.c.; also, Macleod and Robison (*Biochem. J.*,

1929, 23, 517) have shown that the oxidation of reducing sugars by hypiodite can be satisfactorily carried out in solutions of similar concentration. The Hanes modification of the Hagedorn and Jensen method with alkaline ferricyanide, when combined with the iodimetric method, has been satisfactorily applied to the determination of small quantities of mixtures of glucose and fructose and of glucose and maltose (concentrations of about 3 mgrms. per 5 c.c.). In the oxidation of sugars by alkaline ferricyanide a cooling procedure is adopted to prevent loss of iodine and consequent introduction of serious error. The methods have also been applied to the determination of the glucose and maltose obtained by the hydrolysis of starch by taka-diastrase. Tests with pure starch showed that the method was accurate to within 1 per cent. It has been shown that there is no loss of sugar when a solution of glucose and maltose is cleared with basic lead acetate and sodium phosphate. It has been shown that cleared, coloured solutions obtained from alcoholic extracts of young apples should be boiled with a preparation of charcoal such as "Suchar" before determinations of the fructose and glucose by oxidation with alkaline ferricyanide and hypiodite are made. The combination of the two methods of oxidation appears to give satisfactory results for the determination of small quantities of glucose and fructose and glucose and maltose, and could probably be applied to mixtures of any two sugars, provided that the ratio between the factors for the two sugars given by one method of oxidation differs sufficiently from the corresponding ratio given by the other method. The method is convenient, since the same thiosulphate solution is used for both sets of titrations.

P. H. P.

**Composition of Karité Butter.** J. Bougault and G. Schuster. (*Compt. rend.*, 1931, 193, 362-364.)—Two samples of different origin gave acid value, 0.67 to 0.73 per cent. (as palmitic acid); iodine value, 44 to 46; saponification value, 200; unsaponifiable matter, 1.3 per cent. They were examined by the method of Hilditch (oxidation with potassium permanganate in acetone solution, cf. *ANALYST*, 1931, 406), which left unattacked 7.3 per cent. of totally-saturated glycerides made up of tributyrin (3.10), dibutyro-stearin (3.10), and arachido-dipalmitin (0.96 per cent.); separation was achieved by fractional crystallisation from alcohol at 0° C., then from mixed ether and acetone, and finally from petroleum spirit. The 92.7 per cent. of incompletely-saturated glycerides were oxidised to glycerides which were separated by fractional crystallisation and were shown to contain at least one molecule of azelaic acid. The results indicated the presence of dipalmito-olein (19), dibutyrolein (54), and palmito-diolein (19 per cent.) in the original fat. Small amounts of other glycerides may also be present, but the data given, which are only approximate, leave room for not more than 5 per cent. of other substances.

J. G.

**Expressed Brazil Nut Oil.** H. A. Schuette and W. W. F. Enz. (*J. Amer. Chem. Soc.*, 1931, 53, 2756-2758.)—The value of the oil from the seeds of *Bertholletia excelsa* depends upon the extraction process used. Virgin oil, colourless to pale yellow, may be obtained by expression, whereas extraction of the residual

pulp by solvents will yield a darker inferior product, and, although the superior oil solidifies the more readily, a detailed examination of the saturated and unsaturated acid fractions shows that the linolin and palmitin contents of both grades are of the same order of magnitude. The approximate percentage composition of virgin Brazil nut oil was: Myristin, 0.48; palmitin, 13.74; stearin, 5.45; olein, 42.79; and linolin, 26.54. Myristin is present in the virgin oil only to one-fourth of the proportion in the lower-grade product, but there is twice as much stearin.

D. G. H.

**Kapok Oil and the Halphen Test.** H. P. Trevithick and W. H. Dickhart. (*Oil and Fat Ind.*, 1931, 8, 305 and 317.)—A sample of kapok oil, crushed from imported seeds, gave the following analytical results:—Moisture and volatile matter, 0.45; insoluble impurities (meal), 0.36; unsaponifiable matter, 0.66; free fatty acids (oleic), 12.13; and refining loss, 35 per cent.; sp. gr. at 15.5° C., 0.9221; iodine value (Wijs), 94.9; saponification value, 194.5; colour (refined oil) 35 yellow, 7 red; titre (refined oil), 28.1° C.; titre (soapstock acids), 30.2° C.; Halphen test, strong and immediate. Owing to the similarity of this oil to cottonseed oil, tests were made to determine the effects, on the Halphen reaction, of adding each oil in small proportions to sesame, olive, palm kernel, and coconut oils. In every case the colour developed in this reaction by kapok oil was much deeper than that caused by the same percentage contamination with cottonseed oil. The colour obtained with any mixture containing kapok oil in a certain proportion is equivalent to that furnished by a mixture containing ten or more times such proportion of cottonseed oil. Mixtures of oils containing either 5 per cent. (or more) of cottonseed oil or 1 per cent. (or more) of kapok oil yield Halphen colorations which are too deep to allow the percentage contamination to be estimated.

T. H. P.

**Tonka-bean Oil.** G. D. V. Georgi and G. L. Teik. (*J. Soc. Chem. Ind.*, 1931, 50, 318T.)—*Dipteryx odorata*, which yields tonka beans, is indigenous to S. America, and has recently been introduced into Malaya, where the conditions appear suitable to it. A sample of the beans showed the average weight per fresh bean to be 3.4 grms. or per fresh kernel 3.2 grms. The kernels contained: Moisture (loss at 100° C.) 43.1 per cent., and oil (petroleum spirit extract) 26.5 per cent. The oil began to melt at 7.2° C., and was completely fused at 11.8° C.; sp. gr. at 99°/15.5° C. 0.878; saponification value, 198.5; iodine value (Wijs), 72.6;  $n_D^{25}$ , 1.4680; acid value, 1.0; and unsaponifiable matter, 0.5 per cent. The fatty acids had the solidifying point (titre value), 42.0° C.; mean molecular weight 302.9, and iodine value (Wijs) 76.9. Partly on the basis of the high saponification value, 257, given by Duyk (*Répert. Pharm.*, 1908, 193), the oil has previously been classified with the coconut oil group, but the above results, showing normal saponification and low iodine values, indicate that it should be regarded as a non-drying oil. Since coumarin can be manufactured synthetically, and since there are available for edible purposes enormous quantities of other oils of the same class, e.g. arachis oil, extensive demand for tonka beans seems unlikely.

T. H. P.

**Oil from the Seeds of *Putranjiva Roxburghii*, Wall. S. Krishna and S. V. Puntumbekar.** (*J. Indian Chem. Soc.*, 1931, 8, 301–306.)—The putranjiva or jiputa tree (*Putranjiva Roxburghii*, Wall., N. O. *Euphorbiaceae*) is an evergreen tree of tropical India, where in certain parts the leaves and stones of the fruit are officinal and given in decoctions for colds and fevers. The stones of the ripe fruits contained 46 per cent. of kernels, yielding 25 per cent. of oil, that obtained by cold expression being light yellow and clear, but deepening to orange on keeping, with deposition of a fine curdy precipitate. The values obtained from a sample of freshly extracted oil (1) and one extracted in 1925 (2) were as follows:—Sp. gr. at 30°/30° C., 0.9108, 0.9233;  $n_D^{25}$ , 1.4615, 1.4623; saponification value, 189.9, 198.5; iodine value (Hanus), 93.8, 79.49; acetyl value, 9.5, 43.3; Hehner value, 95.4, 96.2; unsaponifiable matter, 0.75, 0.73 per cent.; acid value, 0.9, 11.13. The fatty acids were separated by Twitchell's lead salt and alcohol method, and the groups of mainly-saturated and mainly-unsaturated acids were converted into the methyl esters, which were systematically fractionated. The mixed fatty acids had a mean molecular weight of 292.5, and an iodine value (Hanus) of 101.9, and consisted of 21.4 per cent. of saturated, and 78.6 per cent. of unsaturated acids. The largest of the ester fractions of the unsaturated acids were hydrolysed, the soaps oxidised with potassium permanganate in dilute cold solution, and the hydroxy acids (obtained by neutralising the mixture with sulphur dioxide) were extracted with petroleum spirit. Various compounds, regarded as isomeric acids, of the formula  $C_{18}H_{36}O_6$  were obtained. A portion of the liquid acids was brominated and found to consist of 80 per cent. of oleic acid and 20 per cent. of linolic acid. The methyl esters of the saturated acids were also fractionated, and stearic and lignoceric acids were identified. The phytosterol of the unsaponifiable matter was identified as sitosterol.

D. G. H.

**Determination of Iodine and Chlorine in Iodised Oils. T. T. Cocking and G. Middleton.** (*Quart. J. Pharm.*, 1931, 4, 175–177.)—Commercial iodised oils are normally prepared by the action on a fixed oil containing unsaturated fatty acid radicals (usually sesame oil) of solutions of iodine chloride in glacial acetic acid or of iodine and mercuric chloride in strong alcohol. Absorption of halogens is additive in the dark, but some substitution occurs in sunlight, and, since the 2 halogens are seldom present in equivalent proportions, the total halogen content does not enable the amount of iodine to be obtained. If 1 gm. of oil is boiled under a reflux condenser for 1 hour with 10 c.c. of glacial acetic acid and 1 gm. of zinc filings, the halogens are converted into zinc salts. The condenser is then washed down with 30 c.c. of hot water, and the mixture filtered through cotton-wool, two 20 c.c. portions of water being used as washings. The combined cold filtrates are titrated with 0.05 *M* potassium iodate solution (1 c.c.  $\equiv$  0.01269 gm. I) in the presence of 100 c.c. of concentrated hydrochloric acid, a few c.c. of chloroform being added when the solution becomes light brown, and the end-point being taken when, on shaking, the chloroform is colourless and the aqueous layer yellow. The total halogens (and thence, by difference, the chlorine) are determined by



titration of the acid aqueous liquid with silver nitrate (Volhard). Agreement to within 0.2 per cent. of iodine was obtained by comparison against Middleton's method (ignition with alkali and oxidation to iodate, see *id.*, 1929, 2, 536). A mixture of sesame oil and Wijs solution allowed to react for 1 hour was also shown by the authors' method to contain as much iodine (to within  $\pm 0.001$  grm.) as the Wijs solution alone. J. G.

**Non-Volatile Organic Acids in Barley, Maize, Oats and Rye Plants.** E. K. Nelson and H. H. Mottern. (*J. Amer. Chem. Soc.*, 1931, 53, 3046-3048.)—The cereal plants used in these investigations were cut when they were beginning to form heads. The non-volatile acids were isolated as lead salts and then converted into ethyl esters, which were separated by fractional distillation. Barley plants were found to contain aconitic, malic, citric, malonic, tricarballic and oxalic acids. The oxalic acid, calculated on the fresh material, amounted to 0.019 per cent. Maize plants contained aconitic, malic, citric, tricarballic and oxalic acids. The oxalic acid was found to be 0.029 per cent. (on the fresh plants). Oat plants contained aconitic, malic, citric, malonic, and oxalic acids. The amount of oxalic acid calculated on the fresh material was 0.04 per cent. Rye plants contained aconitic, malic, citric and oxalic acids. The oxalic acid amounted to 0.048 per cent. The occurrence of aconitic acid in these cereals, as well as in sugar cane and sorghum, is noteworthy.

**Detection of Bilberry Juice by means of Plahl's Reaction modified for Use with Sweet Wines.** R. Ofner. (*Chem. Ztg.*, 1931, 69, 666.)—Plahl's reaction (*ANALYST*, 1907, 32, 92; 1908, 33, 191) is suitable for the detection of bilberry juice only in completely fermented wines, and is modified as follows for sweet wines:—The residue from the alcohol or volatile acid determination (or 100 c.c. of the sample) is warmed with 5 grms. of decolorising charcoal for 5 minutes, evaporated to half its volume on the water-bath, diluted to 100 c.c., and filtered by suction. The sugars are removed by 6 washings with hot water, and the residual charcoal heated for 2 minutes, with shaking, with 100 c.c. of water and 5 c.c. of 2 *N* sodium hydroxide solution. The brown filtrate from the cooled liquid (40 c.c.) is shaken with 2 c.c. of *N* hydrochloric acid and 2 c.c. of lead acetate solution and filtered. The filtrate, which is still alkaline, should be colourless, otherwise the treatment is repeated, 10 c.c. of the final filtrate being mixed with 1 c.c. of concentrated hydrochloric acid. No colour results, but, on warming for 15 seconds, the lead chloride dissolves, and, in the presence of bilberry juice, a blue colour appears. The test may be applied in conjunction with Werder's (sorbitol) fruit wine test, in which charcoal is also used. J. G.

**Colorimetric Method for Determination of the Preservative Value of Hops.** J. M. French. (*J. Inst. Brewing*, 1931, 37, 436-439.)—The coloured solutions of the lead salt of humulon, prepared according to Guthrie and Philip's directions (*ANALYST*, 1930, 55, 703), become turbid with certain hops, but this trouble is avoidable by the use of 0.7 per cent. uranium nitrate solution in place of

uranium acetate. The standard solutions made up with uranium acetate in methyl alcohol are found to be unstable in daylight. Hence a series of standard solutions was prepared, with contents of 0.005, 0.0055, 0.006, 0.0065, 0.007, 0.0075, 0.008, 0.0085, and 0.009 grm., respectively, of the  $\alpha$ -resin in 100 c.c. of re-distilled industrial methylated spirit (the first 150 c.c. of distillate from 1 litre being discarded). Ferric chloride solutions to match the colours of this standard series were then made up and have been retained as the standards for use in the test.

The procedure has been simplified as follows:—Ten grms. of the finely minced hops are extracted with 100 c.c. of redistilled methylated spirit for 10 minutes in a corked bottle, with occasional shaking. The hops are allowed to settle, and 0.5 c.c. of the liquid is transferred by means of a graduated 1 c.c. pipette into a Nessler tube marked at 50 c.c. About 10 to 15 c.c. of the redistilled alcohol and 7 c.c. of the uranium nitrate solution (0.7 per cent.) are then added, followed by the same alcohol to the mark. The colour of the liquid is matched with that of one of the standard ferric chloride solutions. In comparing the colours of these two solutions, it is found permissible to withdraw a measured quantity from the solution which may be slightly the darker. This method gives results virtually identical with those furnished by Guthrie and Philip's procedure, and allows of the estimation of a difference of one unit in the preservative value. Owing to changes which occur in the hop extract and in the coloured solutions, all determinations must be made as soon after the extraction as possible.

Various grades of pure alcohol and industrial methylated spirit may be used without diminishing the accuracy of the method, provided that one and the same alcohol is used in the test and in preparing the standards. The uranium nitrate solution keeps well in bottles of amber-coloured glass.

T. H. P.

**The Resin of Ipomoea: Mexican Scammony Root: its Solubility in Ether and the Acid Value as a Test for Rosin.** C. E. Corfield and W. R. Rankin. (*Pharm. J.*, 1931, 127, 76.)—Practically the whole of the scammony resin of commerce now comes from the Mexican scammony root (*Ipomoea orizabensis*, Ledanois) and that from *Convolvulus scammonia* is rare. The value of the determination of the ether-soluble solids of the resin is discussed, the test being regarded as useful provided the conditions are fixed. One grm. of resin and 50 ml. of ether (sp. gr. 0.720) are suggested, and the standard should be in the form of a limit test of not more than 40 per cent. of ether-soluble resins. The determination of the acid value is recommended for ensuring the absence of colophony.

D. G. H.

**Assay of Official Balsams.** T. T. Cocking. (*Pharm. J.*, 1931, 127, 73–74.)—The official method for the assay of cinnamic acid in prepared storax fails to extract all the acid, owing to the hardening of the matrix under prolonged boiling, and the consequent protection of the inner portions from complete extraction, and no methods involving the addition of various substances to keep the resinous mass semi-liquid were successful. A simpler modification of the magnesia method for the extraction of balsamic acids is described, whereby the total acids

from the saponified balsams are converted into the corresponding magnesium salts, which are separated from the resin acid salts by filtration. Any incompletely separated aromatic alcohols are separated from an ethereal solution by extraction with a solution of sodium bicarbonate, and the balsamic acids are finally extracted from an acidified solution with ether, and are weighed after drying *in vacuo* over sulphuric acid. With varying preliminary treatment the free balsamic acids in balsam of tolu and benzoin may be similarly determined.

D. G. H.

**Determination of Strychnine in Easton's Syrup.** L. A. Haddock and N. Evers. (*Pharm. J.*, 1931, 127, 72.)—The total alkaloids are dissolved in 20 c.c. of hydrochloric acid, washed into a separator with a further 5 c.c. of acid, followed by 25 c.c. of a saturated solution of sodium chloride, and the acid liquid is extracted by shaking for five minutes with five successive portions of 25 c.c. of chloroform. The chloroform extract is shaken for five minutes with 5 c.c. portions of an equal volume of *N* hydrochloric acid and saturated sodium chloride, and the combined washings extracted with 10 c.c. of chloroform, which are added to the first extract. After shaking with a mixture of 20 c.c. of water and 5 c.c. of 10 per cent. ammonia, the chloroform extract is separated, washed with 5 c.c. of water, the chloroform distilled off, 1 c.c. of alcohol added, the residue left on evaporation is dried at 100° C. The residue in the flask is washed with three portions of 2 c.c. of a mixture of 2 volumes of ether and one volume petroleum spirit previously saturated with strychnine, the solvent being decanted each time through cotton wool, and any alkaloids on the wool are washed back into the flask with 3 c.c. of chloroform. One c.c. of alcohol is added, the liquid evaporated, and the residue of strychnine dried and weighed. If the original syrup is used, 50 c.c. of syrup are taken with 50 c.c. of saturated sodium chloride solution and 5 c.c. of concentrated hydrochloric acid, and double quantities of chloroform throughout the process.

D. G. H.

**Quantitative Methylation of Theobromine and Theophylline, and the Determination of these Substances in Theobromine Sodium Salicylate and Theophylline Sodium Acetate.** P. A. W. Self and W. R. Rankin. (*Pharm. J.*, 1931, 127, 75–76.)—A method is described whereby 99.5 per cent. of caffeine may be obtained from theobromine and 98.0 per cent. from theophylline. Theobromine may be determined in theobromine sodium salicylate by dissolving 1 gm. of theobromine sodium salicylate in 10 c.c. of water, adding 2 c.c. of *N* sodium hydroxide and 0.6 c.c. of dimethyl sulphate and shaking for 5 minutes. After 30 minutes 3 c.c. of *N* sodium hydroxide solution are added, the mixture shaken for 1 to 2 minutes, transferred to a separator with chloroform and a little water, and the caffeine extracted by shaking with successive portions of chloroform, and washing each extract with about 10 c.c. of water. The chloroform is evaporated, and the residue of caffeine dried at 100° C. and weighed; each gm. of anhydrous caffeine is equivalent to 0.9278 gm. of theobromine. Theophylline may be determined in theophylline sodium acetate by mixing 1 gm. of the substance with 4 c.c. of *N* sodium hydroxide solution, 5 c.c. of water and 0.8 c.c. of

dimethyl sulphate, shaking for 5 minutes or until a clear solution is obtained, and leaving for 1 hour with frequent shaking, when 3 c.c. of *N* sodium hydroxide solution are added, the mixture shaken for 2 minutes, the caffeine extracted as described above and the same factor used for conversion to anhydrous theophylline.

D. G. H.

**Theophylline Sodium Acetate of Commerce.** G. J. W. Ferry. (*Pharm. J.*, 1931, 127, 74-75.)—Twelve samples offered as theophylline sodium acetate were analysed qualitatively and quantitatively; only three actually consisted of this compound, which should contain not less than 60 per cent. of anhydrous theophylline, and not more than 5 per cent. of water. The examination of theophylline sodium acetate is discussed with particular reference to its distinction from theobromine sodium acetate and to variations in composition, since many samples are deficient in theophylline, owing mainly to the presence of excess of water. Neither the formula usually given for the anhydrous salt nor that for the monohydrate accurately represents the actual composition of most specimens, and the introduction into the British Pharmaceutical Codex of monographs on theophylline and theophylline sodium acetate is recommended.

D. G. H.

**Colorimetric Evaluation of *Folia Digitalis* according to the Method of Knudson and Dresbach.** B. J. Okeloan and J. C. Timmers. (*Pharm. Weekblad*, 1931, 68, 820-824.)—Ten c.c. of an infusion of the powdered leaves, 10 c.c. of water and 2.5 c.c. of a 10 per cent. solution of neutral lead acetate are thoroughly mixed, made up to 25 c.c. in a flask, filtered, and 12.5 c.c. of the filtrate precipitated with 1.25 c.c. of a 10 per cent. solution of (crystalline) disodium hydrogen phosphate. The new mixture is also diluted to 25 c.c. and filtered, and 5 c.c. of the bright filtrate are pipetted into a small tube (diameter 20 mm.). A mixture of 95 c.c. of 1 per cent. picric acid and 5 c.c. of 10 per cent. sodium hydroxide solutions is then added from a burette until the colour, which changes from yellow to orange, reaches a maximum and constant degree of intensity. After 25 to 40 minutes the colour may be matched against that produced from 5 c.c. of a standard ouabain solution by 5 c.c. of the picric acid reagent according to the method of Knudson and Dresbach (*ANALYST*, 1923, 48, 76), but any error due to the uncertain strength of this standard is avoided if a solution of potassium dichromate is used alone. This may be titrated with 0.1 *N* sodium thiosulphate solution after liberation of iodine by addition (to 10 c.c.) of 5 c.c. of *N* sulphuric acid and 0.5 gm. of potassium iodide. Five, 3, and 1 per cent. infusions of a sample having a Focke physiological value of 4 (Baljet, *Chem. Weekblad*, 1918, 55, 457) required 15.2, 9.2 and 3.0 c.c. of thiosulphate, respectively. Diffused daylight is preferable, and artificial light is unsuitable, for matching purposes. Ten samples, including *D. ambigua*, *lanata*, *lutea*, *purpurea* (1929-season), gave results showing a maximum divergence from the Focke values (4 to 9) of 0.5; the *D. lanata*, which had a Focke value of 18.2, gave a colorimetric value of 17.1. The Martindale-Westcott method was found suitable for identifying these varieties. Addition of 100 mgrms. of dextrose, laevulose, sucrose, lactose, mannose, maltose and arabinose

to 5 c.c. of picric acid reagent gave a negative colour reaction in the cold in all cases except for laevulose, and positive on warming in all cases except for maltose.  
J. G.

## Biochemical.

**Blackening of Potatoes after Cooking.** C. K. Tinkler. (*Biochem. J.*, 1931, 25, 773-776.)—Potatoes sometimes darken considerably on the surface after cooking. This darkening is distinct from that which occurs with raw potato on exposure to air, and is a matter of considerable importance to those concerned in the growing and sale of potatoes. A simple test has been devised by means of which it is considered possible to tell by examination of a raw potato whether or not a similar potato will blacken after cooking. It was found that all potatoes examined contain a substance (or substances) in greatly varying amounts, which on treatment with nitrous acid followed by an alkali, gives a fine red colour. The amount of the red substance produced in this test was found to vary exactly with the amount of blackening which takes place on cooking. The test is as follows:—A transverse section of potato, about 5 mm. thick, is peeled thinly and covered with 7 per cent. sodium nitrite solution (about 25 c.c.) in a small porcelain basin. About 2 c.c. of dilute hydrochloric acid (1 volume of concentrated hydrochloric acid to 2 volumes of water) are added, and the mixture left for 5 minutes. The liquid is then poured off and the section of potato covered with 16 per cent. sodium hydroxide solution (about 25 c.c.). The red colour develops in about 5 minutes, at first chiefly on the outer and inner edges of the fibro-vascular layer of the potato, then through the whole of this layer, but it often extends towards the centre. It is strongly marked where there are eyes in any potato. After some time the coloured substance is partly extracted by the sodium hydroxide solution. The reaction has not yet been investigated fully. It may be that the nitrous acid reacts with a primary amino-compound, and that coupling of the diazo-compound thus produced takes place with a phenoxide on the addition of the sodium hydroxide, or the colour may be due partly or entirely to reactions between the nitrous acid and lignocellulose. It seems probable that the production of the colour in the test described is in some way connected with the blackening which takes place after cooking, for if the fibro-vascular layer of a potato which would darken, which gives most colour in the test, is completely removed before cooking, very little blackening of the remainder is usually noted after cooking. It is extremely probable that the blackening is due to oxidation; its cause as distinct from darkening due to enzymic oxidation is discussed, and attention is drawn to the fact that iron may have a pronounced influence on the degree of blackening observed.  
P. H. P.

**Free and Bound Water Determinations by the Heat of Fusion of Ice Method.** W. Robinson. (*J. Biol. Chem.*, 1931, 92, 699-709.)—The dissolved materials and the colloids which are present in the water of the tissues have the effect of modifying some of the properties of the water in which they occur; consequently the water of the tissues does not behave in all respects like water in its

pure state, and it is commonly spoken of as "bound" water. Any variations in the concentration of substances in solution and any changes in the water-binding capacity of the colloids, which take place as a result of the processes of normal physiology or of pathology, may affect the degree of force with which the water is bound. The conception has been held by some investigators in this field that part of the water in the tissues is bound and the remainder free, and that an equilibrium is maintained between these two conditions. According to other theories (Briggs, unpublished data, 1931) all the water in the tissues is bound and changing conditions affect only the force with which it is bound. Whichever view is adopted, the method of Rubner (*Abhandl. Preuss. Akad., Physik-Math. Klasse*, No. 1, 1 (1922)) for bound water determinations can be applied to any series of comparative tests. The colloids and dissolved materials hold water with an actual tenacity and resist any force which tends to pull the water away. In Rubner's method a definite and constant desiccating force is used which withdraws the "free" water and leaves the "bound." The method for free and bound water determinations, which is described in detail, is modified from the description given by Thoenes (*Biochem. Z.*, 1925, 157, 174) and by Robinson (Colloid Symposium Monograph, 1928, 5, 199), but does not differ materially from the principles of the method of Rubner. A known weight of specimen, preferably between 0.4 and 0.8 grm., is placed in a prepared tin-foil container of known weight, frozen at a constant temperature of  $-20^{\circ}\text{C}$ . for several hours, and transferred to a calorimeter where a determination is made of the number of calories required to melt the ice formed within the tissues (based upon the fact that to melt 1 grm. of ice without raising its temperature requires 80 calories). By calculation the amount of free water per grm. of solid is determined. For the final step in the method the material is dried to constant weight as a measurement of total water content. The difference between the total and the free water values indicates the amount of bound water in the specimen. In the freezing process an efficient desiccating force is provided, since at temperatures below zero water tends to leave the colloids and dissolved materials to form ice crystals of pure water. The force exerted by this means is definite and constant at any given temperature below the freezing point of the tissue, and increases with fall in temperature, but the tendency of the water to crystallise is retarded by the dissolved and colloidal substances present. Therefore, at any given temperature below freezing the amount of water which crystallises out is a measure of the water-binding capacity of the tissue. With the use of the technique described, results with homogeneous material may be obtained which are reproducible within a small range of error. The number of data necessary for each determination makes a separate work sheet essential for each specimen, and the form of that used by the author is shown. The formulae necessary for the calculations are given. P. H. P.

**Simplification of Okey Method for Determination of Cholesterol by Oxidation of the Digitonide.** M. E. Turner. (*J. Biol. Chem.*, 1931, 92, 495-498.)—The method of Okey (*J. Biol. Chem.*, 1930, 88, 367; *ANALYST*, 1930, 55,

654) for the determination of cholesterol by means of oxidation of the digitonide is an intricate procedure, especially in the precipitation of the cholesterol digitonide. The need for special and difficultly obtainable apparatus and for practice in the technique of making numerous transfers and filtering and washing small amounts of material discourages general application of the method. A simplification has been developed in which the precipitation, washing, and oxidation are accomplished in the one centrifuge tube (a 15 c.c. centrifuge tube with conical bottom and with well-fitted ground stopper). A table shows the figures for the recovery of free cholesterol from artificial and natural plasma extracts, as well as comparison figures with the colorimetric method of Autenrieth and Funk (*Münch. med. Woch.*, 1913, 69, 1243). The limits of error in the oxidative method are +2 to +4 per cent. for 0.5 to 1.5 mgrm. of free cholesterol, and in the colorimetric method +8 to +12 per cent. The method described is applicable to the determination of free and combined cholesterol, and also lends itself to use in the determination of sterols of those plant extracts for which the colorimetric methods cannot be used.

P. H. P.

**Activation of Ergosterol with Radium Emanation.** R. B. Moore and T. De Vries. (*J. Amer. Chem. Soc.*, 1931, 53, 2676-2681.)—A sample of reasonably pure ergosterol prepared in the United States (m.pt., 148° C., specific rotation in chloroform  $-100^\circ$ ), and one from Germany (m.pt., 157° C., specific rotation in chloroform  $-121^\circ$ ) were activated with radium emanation on four different occasions, and the activated samples were tested on rachitic white rats. Results showed that the degree of potency obtained was about 0.01 of that of a good grade of ergosterol radiated with ultra-violet light, and the activation of the samples appeared to be permanent. Stirring the sample during activation increased the speed of activation, but not appreciably the potency reached, and a twenty-fold excess of activation caused no appreciable loss in potency; some decomposition products were, however, present in all the experiments.

D. G. H.

**Influence of Solvents on the Activation of Ergosterol.** C. E. Bills, E. M. Honeywell and W. M. Cox, Jr. (*J. Biol. Chem.*, 1931, 92, 601-604.)—Previously described biological and spectrographic measurements made on alcoholic solutions of ergosterol after different periods of irradiation have shown that the quantity of vitamin D in the photochemical reaction product rapidly rises to a maximum and gradually declines to zero. The spectrographic changes are no measure of anti-rachitic potency, and thus, to gain knowledge of the rise and decline of potency, it is usually necessary to prepare an activation curve. The authors have investigated the influence of solvents on the activation of ergosterol, with the use of cyclohexane and ether in comparison with alcohol. At least 100 rats were used for each activation curve, so that the probable error for the curves was only  $\pm 4$  per cent. The three curves, which are given, had the same general shape, but widely different dimensions. Of the three solvents, ether permitted by far the greatest activation. With alcohol, the maximum cod-liver oil coefficient was reached in the shortest time, 22.5 minutes, but the maximum

was the lowest (250,000), and the decline in potency was the most rapid. After 3 hours and 18 minutes all antirachitic activity disappeared. With cyclohexane, the maximum was reached in 27 minutes, but the maximum was somewhat higher (330,000). The decline was much more gradual; the reaction product showed a cod-liver oil coefficient of 25,000 even after 14 hours of exposure. With ether the maximum was reached in 4 hours, 12 minutes, but the maximum was by far the highest (710,000). The decline in potency was relatively more rapid than with cyclohexane, yet even after 18 hours of exposure the reaction product showed a cod-liver oil coefficient of 25,000. In ether the attainment of maximum potency required an hour more of exposure than was required in alcohol for the entire cycle of rise and decline. The authors have no satisfactory explanation of the observed influence of solvents on activation. The three solvents used are transparent to the short wave-length side of the ergosterol absorption curve, and each, when pure, transmits a high percentage of radiations between  $230\mu$  and the visible region, and hence might be expected to give similar results under similar conditions of exposure. One might presume that the different solvents had different protective, anti-oxidative actions.

P. H. P.

**Experiments on Nutrition. X. Comparative Vitamin  $B_1$  Values of Foodstuffs. Cereals II.** R. H. A. Plimmer, W. H. Raymond and J. Lowndes. (*Biochem. J.*, 1931, **25**, 691-704.)—In previous experiments by Plimmer, Rosedale, Raymond, and Lowndes (*Biochem. J.*, 1927, **21**, 114) on the testing of cereals for their vitamin  $B$  ( $B_1$ ) value, rearing of young by pairs of adult pigeons was taken as the standard of comparison. It was shown that a greater amount of foodstuff containing vitamin  $B$  was needed for rearing than for maintenance, and this fact has been again observed in subsequent experiments. Therefore the standard of maintenance for a period of at least 26 weeks was adopted with satisfactory results by Plimmer, Raymond and Lowndes (*Biochem. J.*, 1929, **23**, 546) in their trials with pulses and nuts. In order to bring cereals into proper comparison it was necessary to test them again on this standard, and the results obtained are given. Some experiments were also made on the extraction of vitamin  $B$  from wheat germ, and marmite was again tested for comparison with the cereals, and for ascertaining if its value is the same after a period of 5 or 6 years. There is no essential change in the vitamin  $B$  value of marmite in a period of 6 years; it may lose in value if kept for long periods, but freshly manufactured marmite seems always to have approximately the same value. The term "vitamin  $B$ " is now used to include several unknown factors. In the tests the symptoms of head retraction of pigeons were taken as the sign of insufficient vitamin  $B$ , i.e. vitamin  $B_1$  in the modern nomenclature. With the use of the same standard of comparison as in the work on pulses and nuts, the comparative vitamin  $B$  values of cereals, etc., are as follows:

				Percentage amount in diet for maintenance.	Comparative vitamin $B$ value.
Dried yeast	..	..	..	4	100
Marmite	..	..	..	6	67



	Percentage amount in diet for maintenance.	Comparative vitamin B value.
Wheat germ ("bemax") ..	6-7	62
Middlings .. ..	10	40
Baker's yeast .. ..	12	33
Bran .. ..	20	20
Buckwheat .. ..	20	20
Millet .. ..	30	13
Oatmeal .. ..	35	11
Wheat .. ..	40	10
Barley .. ..	40	10
Malt .. ..	40	10
Rye .. ..	40	10
Dari .. ..	40	10
Brown rice .. ..	40	10

Cereals are thus not quite so rich in vitamin B as pulses, which have a comparative value of 13. P. H. P.

**Investigations on Vitamin B<sub>2</sub>. I. Sources of Vitamin B<sub>2</sub>. II. Stability of Vitamin B<sub>2</sub>. III. Chemistry of Vitamin B<sub>2</sub>. B. C. Guha. (Biochem. J., 1931, 25, 945-959.)**—The values as sources of vitamin B<sub>2</sub> have been investigated of milk powder and aqueous extracts of brewer's yeast, baker's yeast, fresh ox-liver, beef muscle and Eli Lilly's liver concentrate No. 343. The fresh ox-liver extract is apparently the most potent of those. An aqueous extract (20 per cent.) of the liver concentrate is also very potent, being effective in a daily dose of 40 to 60 mgrms. for the growth of young rats. The advantages of this liver concentrate over yeast as a source of vitamin B<sub>2</sub> are pointed out; it can be readily prepared from a stock of the solid liver concentrate whenever required. The stability of vitamin B<sub>2</sub> preparations obtained from different sources towards heat and alkali shows curious discrepancies. The vitamin B<sub>2</sub> in aqueous extracts of yeast and fresh ox-liver is much less stable than that in marmite and in the commercial liver concentrate. Probably the stability of certain preparations is connected with the presence of some kind of protective material in them. An aqueous extract of the liver concentrate, autoclaved at pH 9 for half an hour at 124 to 125° C., provides an excellent source of vitamin B<sub>2</sub> free from vitamin B<sub>1</sub>; it is preferable to autoclaved marmite, for it contains less solid matter and is more palatable to the rats. A chemical study of vitamin B<sub>2</sub> in a cold aqueous extract of the liver concentrate has been made. Picric acid, benzoyl chloride, phosphotungstic acid and flavianic acid do not precipitate the vitamin. Nitrous acid neither precipitates nor inactivates it. Lead acetate and silver nitrate precipitate it partially. Esterification leaves about 40 per cent. of the vitamin in the non-esterified fraction; the esterified fraction is inactive. It is not attacked by trypsin. Norit charcoal adsorbs the vitamin at the normal pH (4.6) of the aqueous extract of the liver concentrate; it was not possible to elute it effectively by aqueous alcohol, 30 per cent. propyl alcohol or dilute saponin solution. The possibility of a relationship between vitamin B<sub>2</sub> and the factor in liver specific for pernicious anaemia is discussed; the chemical behaviour of the vitamin, however, is in contrast to that of

the factor for pernicious anaemia. On the basis of the present evidence it may be provisionally concluded that the vitamin is probably not a base, acid or peptide, but a neutral substance. Preliminary experiments on the electro-dialysis of the vitamin support this tentative conclusion. The partial precipitation by lead acetate and silver nitrate is probably due to the adsorption of the vitamin on the precipitates formed. The vitamin is stable to sulphur dioxide, hydrogen peroxide and ozone. Some of the rats which were maintained at a weight between 60 and 80 grms. over periods of 12 weeks or so, and were not undergoing a drastic deprivation of vitamin  $B_2$ , developed a curious form of depilation, which was cured by administration of the liver-extract. Haemin, haemoglobin and lactalbumin could not ameliorate the above symptoms or produce growth in absence of vitamin  $B_2$ .

P. H. P.

**Quantitative Method for Determination of Vitamin C.** K. M. Key and G. K. Elphick. (*Biochem. J.*, 1931, **25**, 888–897.)—A method is described for the determination of the antiscorbutic potency of a substance in terms of the potency of a standard such as lemon juice or orange juice. Different doses of orange juice were given to a series of guinea-pigs and the amount of protection from scurvy produced in each animal was determined by means of an arbitrary scale. The test on the animals was continued for 14 days, when the growth response, *post-mortem* observations and structure of the roots of the incisors were examined, but the method finally used was based solely on the histological appearance of the roots of the incisors. A curve was constructed relating the average amount of protection afforded to the dose of orange juice given. A straight line was obtained, and this was used as a curve of reference for evaluating results on an unknown substance. Some disadvantages of the use of decitrated lemon juice as a standard are pointed out. Evidence is given which suggests that the anti-scorbutic potency of orange juice is constant, and orange juice is palatable to guinea-pigs and can be given to them directly without previous treatment. The method gives results more accurate than those obtained from the minimum protective dose method, and is useful for determinations of the antiscorbutic potency of substances containing little vitamin C. Satisfactory results, which were obtained in experiments on Bramley's Seedling apples and tomato juice of unknown strength, are recorded.

P. H. P.

**Influence of the Ration of the Cow upon Vitamin B and Vitamin G Content of Milk.** C. H. Hunt and W. E. Krauss. (*J. Biol. Chem.*, 1931, **92**, 631–638.)—It was shown by Hunt and Krauss (*J. Biol. Chem.*, 1928, **79**, 733; *ANALYST*, 1928, **53**, 668) that milk contains a relatively large amount of vitamin G ( $B_2$ ), whilst the amount of vitamin B ( $B_1$ ) is small. Sherman and Axtmayer (*J. Biol. Chem.*, 1927, **75**, 207; *ANALYST*, 1927, **52**, 721) found that dried skim milk was apparently richer in vitamin G than in vitamin B. Aykroyd and Roscoe (*Biochem. J.*, 1929, **23**, 483) made the observation that milk was a poorer source of the antineuritic vitamin than of the antipellagric factor. In view of these observations it was decided to study the influence of the ration of the cow upon the

relative potency of milk in these two known factors of the vitamin *B* complex. The milk was tested on rats. Two experiments were carried out: (1) A comparison of pasture grass with dry feed as to their effect on the vitamin *B* and vitamin *G* content of milk; (2) the influence of the stages of maturity of pasture grasses upon the vitamin *B* and vitamin *G* content of milk. The vitamin *B* and vitamin *G* content of the pasture grasses was also investigated. The results show that milk from cows on pasture has a higher vitamin *G* content than milk from cows on dry feed, although the quality of the hay used in dry feeding may be a determining factor. The vitamin *B* content of the milk is not so affected. Cows on early pasture during its vigorous state of growth produce milk higher in vitamin *G* than do cows on an over-mature pasture. Whilst the vitamin *B* content is not so easily affected, there are slight indications that fresh tender grass produces a milk higher in vitamin *B* than does over-mature pasture grass. It appears that vitamin *G* is synthesised during the process of rapid growth, and then is dissipated as the plant matures. On this basis the highest quality of hay would be produced by cutting it while the plant is still immature, and this appears to be in keeping with practical observations and feeding results.

P. H. P.

## Organic Analysis.

**Determination of Small Proportions of Hydrocarbon in Alcohol containing Acetone.** R. W. Hoff. (*J. Soc. Chem. Ind.*, 1931, 50, 242-244T.)—Since most of the grades of alcohol containing hydrocarbons now legalised by the Canadian Government are denatured with wood spirit (e.g. "Specially Denatured Alcohol" or "Grade No. 1 Benzine," and "Fully Denatured Alcohol" or "Grade No. 2 Benzol") the method proposed by Babington and Tingle (*ANALYST*, 1919, 44, 297), which fails in the presence of acetone, cannot be used. The acetone is therefore converted into a non-volatile compound as follows:—(1) A freshly-prepared amalgam, made by immersing 5 grms. of pieces of aluminium sheet in a saturated solution of mercuric chloride, is lightly washed with water and warmed in a 500 c.c.-Kjeldahl flask with 100 c.c. each of the sample and of 2 *N* potassium hydroxide solution for 1 hour on the water-bath under a reflux condenser (minimum length 2 feet). The mixture is then cooled, the condenser rinsed with 20 c.c. of 90 per cent. alcohol, and then with 200 c.c. of water, and the liquid is distilled at the rate of 1 c.c. per minute through a vertical condenser. The hydrocarbon distillate is collected in two 20 c.c. portions in 50 c.c. stoppered ("Eggertz") tubes graduated to 0.1 c.c., 15 c.c. of a 1/6-saturated potassium dichromate solution and 2 c.c. of hydrochloric acid (sp. gr. 1.2) added in each case, and the whole mixed. After about 15 minutes, when the mixture is olive-green in colour, exactly 10 c.c. of petroleum spirit of low b. pt. are added, and the volume of the petroleum layer read after mixing. If the tube is inverted, any greasiness produced in it during the distillation is avoided. The volume of hydrocarbon is obtained by deducting 10 c.c. from the reading, 0.1 c.c. or less being found in the second fraction. In the case of "petroleum benzine" containing 0.5 to 1.5 grm. of acetone per 100 c.c., the

only error recorded was  $-0.05$  per cent. of hydrocarbon (1 to 2 per cent. present). Solvent naphtha from coal tar, however, gives high results, owing to the greater solvent action for dilute alcohol of the higher benzene hydrocarbons. The factor 0.05 then gives results with a maximum error of 0.09 per cent. (2) When the continuous evolution of hydrogen is liable to cause loss of volatile hydrocarbons, the sample (100 c.c.) is freed from pine oil (*e.g.* by distillation) and mixed with more hydroxylamine hydrochloride (*e.g.* 2 grms.) than will combine with the acetone present, and with sufficient potassium hydroxide solution to give a permanent pink colour with phenolphthalein. The mixture is diluted to 400 c.c., distilled, and 40 c.c. of the distillate collected and treated as in (1). The maximum error is  $+0.05$  per cent. of benzene (2 to 2.5 per cent. present). The methods (subject to criticism) are to be adopted for fiscal purposes in Canada. J. G.

**Determination of Small Proportions of Butyl Chloride and Diethyl Phthalate together in Ethyl Alcohol.** R. W. Hoff (*J. Soc. Chem. Ind.*, 1931, 50, 244r.)—The new Canadian Government "Specially Denatured Alcohol, No. 1-F" (Rubbing Alcohol), contains butyl chloride, diethyl phthalate (0.98 c.c. of each per 100 c.c.), brucine sulphate and quassin as denaturants, the solids being present in amounts insufficient to affect the analytical results if the following procedure is used:—One hundred c.c. are distilled from a 500 c.c. Kjeldahl flask at 3 c.c. per minute through a vertical condenser into a similar flask. The first 70 c.c. contains the butyl chloride, which is determined, after dilution to 400 c.c., by the method proposed for benzene in ethyl alcohol by Babington and Tingle (*ANALYST*, 1919, 44, 297). The diethyl phthalate is determined by hydrolysis of the residue by heating on the water-bath under a reflux condenser for 1 hour with 50 c.c. of 0.5 *N* alcoholic potassium hydroxide solution (*i.e.* an excess equivalent to more than twice the amount of ester probably present). The liquid is cooled, diluted and back-titrated with 0.5 *N* sulphuric acid in the presence of phenolphthalein, allowance being made for any blank on the reagents. If 1.126 is the sp. gr. of the phthalate at room-temperature, the volume present is given by (c.c. of 0.5 *N* alkali used  $\times 0.0555/1.126$ ). The results obtained are accurate to within about 0.01 c.c. for the phthalate and 0.07 for butyl chloride (per 100 c.c.). J. G.

**Reduction of Aromatic Ketones and Benzils by Triphenylmagnesium Bromide.** W. E. Bachmann. (*J. Amer. Chem. Soc.*, 1931, 53, 2758–2763.)—The Grignard reagent, triphenylmagnesium bromide, reduces aromatic ketones such as benzophenone, 4-chlorobenzophenone, 4-phenylbenzophenone, 4,4-diphenylbenzophenone, fluorenone, and xanthone, to pinacols. The reaction proceeds through the intermediate formation of radicals, according to the equation:  $RRC=O + (C_6H_5)_3CMgBr \rightarrow RRC-OMgBr + (C_6H_5)_3C\cdot$ . The ketyl radicals then associate to the pinacolate:  $2RRC-OMgBr \rightleftharpoons RRC(OMgBr)(OMgBr)CRR$ . Benzils are reduced by the reagent to the bromo-magnesium salt of stilbene-diols:  $RCOCOR + 2(C_6H_5)_3CMgBr \rightarrow RC(OMgBr)=(OMgBr)CR + 2(C_6H_5)_3C\cdot$ .

W. P. S.

**Anhydrous Distillation Method for the Determination of Mercury in Organic Compounds.** E. P. Fenimore and E. C. Wagner. (*J. Amer. Chem. Soc.*, 1931, 53, 2468–2475.)—A method for the determination of mercury in such compounds as diacetatomercuriphenol, mercury *p*-ditolyl, *n*-butyl mercury mercaptide, mercuric cyanide, etc., consists in decomposing the compound with concentrated sulphuric acid and ammonium or potassium persulphate, distilling the mercury as mercuric chloride in a current of hydrogen chloride, precipitating the metal as zinc mercuric thiocyanate and subsequently determining it iodimetrically. In the presence of iodine, the mercury in the distillate must first be precipitated with zinc-dust, the excess zinc dissolved in hydrochloric acid, the residual amalgam dissolved, and the mercury determined by the iodate method. The presence of chlorine or bromine does not interfere. W. P. S.

**Copper Determination in Organic Matter.** S. Ansbacher, R. E. Remington and F. B. Culp. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 314–317.)—A critical and very detailed study has been made of methods for determining very minute amounts of copper in organic materials generally, *e.g.* milk, fruit, vegetables, oysters, etc. After destruction of the organic matter by wet or dry oxidation depending on the bulk of the sample, precipitation of the copper by hydrogen sulphide in the presence of co-precipitated sulphur under controlled conditions of acidity has been found the most suitable method for separating the smallest traces of copper from other elements, *e.g.* iron, interfering with the final determination, for which a choice of four tested methods is given. *Destruction of Organic Matter. Wet digestion.*—To a small sample, moistened with water, in a Kjeldahl flask, are added 15 c.c. of concentrated sulphuric acid, and the whole heated until the residue becomes black and sulphur trioxide is evolved. After cooling, 5 c.c. of dilute perchloric acid (20 per cent.) and 2 c.c. of nitric acid are added, and the flask again heated until sulphur trioxide fumes are evolved and the liquid is colourless, more nitric and perchloric acids being added, if necessary, and the heating repeated. *Ashing of the sample.*—Larger samples are ashed in a silica dish in an electric muffle at a temperature not exceeding 400° C., followed by evaporation of the residue to dryness with fuming nitric acid; the product is dissolved in 10 c.c. of concentrated sulphuric acid. *Precipitation of the copper as sulphide and its re-solution.*—The acid liquid is transferred to an Erlenmeyer flask, diluted to give an acid concentration of 15 per cent. by volume, and heated to boiling; a few drops of nitric acid are added and hydrogen sulphide passed through the solution until cold. After the precipitate has settled it is filtered off on a small crucible with a porous bottom, washed with very dilute acetic acid saturated with hydrogen sulphide and finally dissolved in fuming nitric acid; this solution is evaporated to dryness (care being taken not to decompose the copper nitrate), and the residue is dissolved in water, giving a neutral solution containing the copper in the sample. *Determination by Xanthate reagent.*—An aliquot part of the solution is transferred to a Nessler tube containing 10 c.c. of potassium ethyl xanthate solution (0.1 per cent.) and water. To a similar tube containing the same quantity of reagent and

water, standard copper solution (about 50γ of copper per c.c.; 1γ=0.001 mgrm.) is added until an approximate match is obtained after the contents of the tubes have been diluted to the same volume with water. The true amount of copper is obtained by preparing six colour standards containing different amounts of copper very close to the amount indicated, and choosing the one nearest in colour to the unknown. The most reliable results are obtained when from 100γ to 200γ of copper are present in the solutions compared. (This method is a modification of Supplee and Bellis's process for copper in milk, *J. Dairy Sci.*, 1922, 5, 455). *Determination of copper with the "Biazzo" reagent.*—An aliquot part of the solution is transferred to a 25 c.c. separating funnel, and into another funnel is run an approximately equivalent amount of standard copper solution (judged from the appearance of the original copper sulphide precipitate). To each of the funnels are added about 10 c.c. of water, 25 drops of glacial acetic acid, 30 drops of pyridine, 3 c.c. of potassium thiocyanate solution (10 per cent.), and 2 c.c. of bromobenzene (b.pt., 154–155° C.), and the mixture is well shaken. Some of the bromobenzene layer is drawn off and the depths of the two colours compared in a colorimeter. This method works best with 50 to 150γ of copper (Note by Abstractor, *cf.* Chalk, *ANALYST*, 1930, 55, 187). *Determination of Copper by Carbamate reagent.*—The directions are the same as for the xanthate method, this reagent being replaced by 10 c.c. of sodium diethyldithiocarbamate solution (0.1 per cent.) (*cf.* Callan and Henderson, *ANALYST*, 1929, 54, 650). A fourth alternative given is a titrimetric method employing "nitroso chromotropic acid" (1, 8-dihydroxy-2-nitroso-3, 6-naphthalene disulphonic acid), for details of which, and the preparation and standardisation of the reagent, the original memoir should be consulted. (*Cf.* Cherbulier and Ansbacher, *Helv. Chim. Acta*, 1930, 13, 187). Good results were obtained by each of the different methods of finishing the determination.

S. G. C.

**Determination of Sodium in Organic Compounds by the Uranyl Acetate Method.** D. L. Tabern and E. F. Shelberg. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 278–279).—The paper is concerned with the application of the now well-known uranyl acetate precipitation method to the analysis of sodium salts of organic acids, the determination of sodium in some of which, more particularly barbiturates, *e.g.* "Nembutal" [sodium ethyl-(1-methylbutyl) barbituric acid], "sodium Neonol" (sodium ethyl-*n*-butyl barbituric acid), and "sodium Amytal" (sodium ethyl-*iso*-amyl barbituric acid) gave inexplicably low results by the ordinary sodium sulphate method. The following process is given:—To 0.1 grm. (containing from 5 to 20 mgrms. of sodium), dissolved in 5 to 10 c.c. of water or alcohol, are added 3 c.c. of reagent for each mgrm. of sodium expected [reagent: uranyl acetate 33 grm.; magnesium acetate, 100 grm.; acetic acid, 20 c.c.; alcohol (90 per cent.), 500 c.c.; water, to make 1000 c.c.; filtered at 20° C. before use]. The beaker is placed in ice-water for half-an-hour with occasional stirring of the liquid, the precipitate is filtered off on a Gooch crucible, washed with 5 to 10 c.c. of the reagent, and finally with alcohol (95 per cent.), and dried at 105° C.; 1 grm. of the precipitate is equivalent to 0.0153 grm. of sodium. The results obtained with a

number of sodium salts of various organic acids compared well with the theoretical values and with those obtained with a special electrometric titration method.

S. G. C.

**Irregularities in Sodium Determination by Sodium Sulphate Method (in Organic Compounds).** G. W. Collins. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 291.)—The determination of sodium in the sodium salts of certain barbituric acids by the direct sulphate method gives low results (*cf.* preceding abstract). Sodium can be determined accurately in these compounds if the aqueous solution of the sample is acidified and the organic radicle is removed by extraction with ether before the evaporation with sulphuric acid. It is concluded that the direct sulphate method gives low results in the presence of the five-carbon side-chain of malonyl urea; no definite explanation of this is put forward.

S. G. C.

**Determination of Silica in Vegetable Substances by Mixed Nitric and Perchloric Acids.** L. Lematte, G. Boinot, E. Kahane and M. Kahane. (*Compt. rend.*, 1931, 192, 1459–1462.)—Organic matter can be conveniently destroyed by heating with a mixture of nitric and perchloric acids, and the silica is left in an insoluble form. The following method has been found suitable for cereals, straw, bran, pine-needles, and sawdust:—A mixture of 20 c.c. of fuming nitric acid (sp. gr. 1.49) and 30 c.c. of perchloric acid (sp. gr. 1.61) is poured on to 5.0 grms. of the sample contained in a 500 c.c. beaker. The oxidising action generally starts in the cold, but the mixture may be heated from the start, care being taken to prevent too abundant frothing. When the nitric acid has been dispelled, as shown by the appearance of white fumes, the heating is continued for 30 minutes (the beaker being covered). After cooling, about 100 c.c. of water are added, the liquid is boiled, and the silica filtered off, calcined and weighed. The table of results shows interesting differences between the silica contents of different kinds of straw, etc.

S. G. C.

*Note.*—The use of a beaker is emphasised because there is a risk of explosion if a flask is used.—EDITOR.

## Inorganic Analysis.

**Diphenylaminesulphonic Acid as an Indicator.** L. A. Sarver and I. M. Kolthoff. (*J. Amer. Chem. Soc.*, 1931, 53, 2902–2905, 2906–2909.)—The properties of diphenylaminesulphonic acid are similar to those of diphenylamine, but it gives a much sharper end-point, as the introduction of the sulphonc group increases the solubility. The new indicator, unlike the base, can be used in the presence of tungstate; its oxidation is very much accelerated by ferrous ion. It is used in the form of its barium salt (3.17 grms. in 1000 c.c. of water). A comparative study of the indicators diphenylamine, diphenylbenzidine, and diphenylaminesulphonic acid was made in the determination of ferrous iron by means of dichromate, and of dichromate and vanadate by means of ferrous sulphate. The very small corrections required to allow for the amount of indicator added are tabulated. Preference is given to diphenylaminesulphonic acid on account of

its rapid, brilliant, and reversible colour changes, which are unaffected by tungstate. This property is of importance in connection with the analysis of alloy steels.

W. R. S.

**Inaccuracy in the Determination of Mercury by Direct Precipitation as Mercury Sulphide from Acid Solution.** E. P. Fenimore and E. C. Wagner. (*J. Amer. Chem. Soc.*, 1931, 53, 2453–2456.)—Direct precipitation of mercury from an acid solution by means of hydrogen sulphide yields an impure precipitate and the results obtained are too high. Under the most favourable conditions the error may be 0.34 per cent., and this error is increased by the presence of salts in the solution, particularly by the presence of iodides. Volhard's method is accurate with solutions containing little else than mercury, but in the presence of salts it also gives results which are too high.

W. P. S.

**Quantitative Separation of Copper and Cadmium by Reduction with Potassium Formate.** E. I. Fulmer. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 257–258.)—On evaporation of a solution containing copper sulphate and cadmium sulphate (2 grms.) and potassium formate (15 grms.) to dryness and heating the residue at 155° to 160° C. for 1½ hours, the copper is liberated as metal, and the cadmium remains in a form which can be dissolved in water. In a test of the process, good results for copper were obtained by filtering the aqueous extract of the residue and weighing the copper remaining as metal; the results for cadmium are not stated.

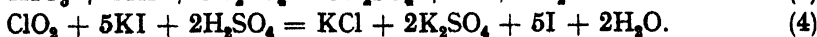
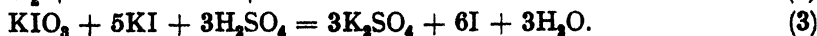
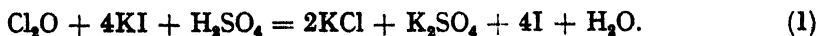
S. G. C.

**Volumetric Determination of Chromium and Nickel in the Same Solution.** L. H. James. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 258.)—The method depends on the discovery by Willard and Cake (*J. Ind. Eng. Chem.*, 1919, 11, 480) that boiling perchloric acid oxidises trivalent chromium to the hexavalent state, which can be titrated by ferrous sulphate in the diluted solution; nickel can then be titrated in the same solution by the well-known Moore cyanide method. *Method for "Chrome-Nickel" and Stainless Steel.*—To 1 gm. of "chrome-nickel" or 0.2 gm. of stainless steel contained in a tall 500 c.c. beaker are added 20 c.c. of an acid mixture containing 250 c.c. of nitric acid (sp. gr. 1.42), 750 c.c. of hydrochloric acid (sp. gr. 1.19) and 1000 c.c. of water; after heating until the material is dissolved, 20 c.c. of perchloric acid (20 per cent.) are added. The solution is boiled until white fumes are given off, and then 10 minutes longer. After cooling, 100 c.c. of water are added, the liquid is boiled for 2 minutes and again cooled. Twenty-five c.c. of dilute sulphuric acid (sp. gr. 1.22) and an excess of 0.1 N ferrous sulphate solution are added, and the unused ferrous sulphate titrated with 0.1 N potassium permanganate solution. The nickel is then determined by the Moore cyanide titration method. The method needs modification for dealing with nickel-chromium iron containing an appreciable percentage of silicon; after the boiling with perchloric acid, which should in this case be continued for 20 minutes, 50 c.c. of water are added after cooling, the silica is filtered off and washed with dilute perchloric acid (1 per cent.). Chromium and nickel are then determined in the filtrate as described above.

S. G. C.



**Analysis of Mixtures of Chlorine Monoxide and Chlorine.** J. W. T. Spinks. (*J. Amer. Chem. Soc.*, 1931, 53, 3015-3016.)—The gas is absorbed in potassium iodide solution, after which the solution is acidified with a known excess of standard acid, and titrated with thiosulphate. This gives the iodine equivalent of chlorine and the monoxide according to equations (1) and (2):



The excess acid over that equivalent to the monoxide according to (1) is then ascertained by reaction (3) after addition of a slight excess of standard iodate solution. Hence the standard acid can be standardised iodimetrically against the same thiosulphate solution. Mixtures of chlorine peroxide and chlorine can be analysed by means of the above method, equation (4) giving the necessary stoichiometric data. W. R. S.

**Iodimetric Titration of Iodide and Nitrite.** C. A. Abeledo and I. M. Kolthoff. (*J. Amer. Chem. Soc.*, 1931, 53, 2893-2897.)—The reaction  $\text{HNO}_3 + \text{HI} = \text{H}_2\text{O} + \text{NO} + \text{I}$  occurs only in acid medium. In the presence of oxygen the nitric oxide is oxidised to nitrogen dioxide, which partly reacts with water, forming nitrous and nitric acids. The conditions for applying the reaction to the accurate determination of iodide and nitrite were ascertained. **Iodide.**—The solution is treated in a glass-stoppered flask with about 1 gm. of urea (to remove nitrous acid and nitric oxide), 5 c.c. of 0.5 *N* sodium nitrite, and 5 c.c. of 4 *N* sulphuric acid, and the flask then stoppered and frequently shaken during 10 minutes. After addition of 1 to 2 grms. of potassium iodide, the solution is titrated with thiosulphate. For very small amounts of iodide, the additions are 0.5 gm. of urea, 1 c.c. of 0.2 *N* nitrite solution, 5 c.c. of chloroform, and 5 c.c. of 4 *N* acid; the stoppered flask is left for 30 minutes before the addition of the iodide and titration. Chlorides do not interfere; if the bromide present exceeds the equivalent of the iodide, results are high. **Nitrite.**—The solution is run into a glass-stoppered flask containing 10 to 12 grms. of sodium bicarbonate, 3 grms. of potassium iodide, 0.5 to 1 c.c. of amyl alcohol (to prevent foaming), and 25 c.c. of water; the ingredients are thoroughly mixed, after which 4 c.c. of glacial acetic acid are distributed, without agitation, through the solution from a pipette. The stopper is loosely inserted, and the flask gently rotated after the evolution of carbon dioxide has nearly ceased. When the bicarbonate has again settled, 6 to 7 c.c. of 20 *N* sulphuric acid are quickly added, and the stopper inserted as before. When most of the gas has escaped the flask is shaken, the stopper rinsed, and the solution titrated with thiosulphate. If in either determination there is a return of the starch-iodine colour, the operation should be repeated. W. R. S.

**Determination of Fluorine.** P. Mougard. (*Compt. rend.*, 1931, 192, 1733-1735.)—Two modifications of the calcium fluoride method for the determination

of fluorine have been studied—(a) Rose's method, in which some calcium carbonate is made to precipitate with the calcium fluoride, and (b) Carrière and Rouanet's method (*Compt. rend.*, 1929, 189, 1281), in which precipitation of calcium fluoride in boiling ammoniacal solution is employed. The subsequent removal of the calcium carbonate from the precipitate in (a), by treatment with acetic acid, is uncertain; some calcium acetate is retained in it, and the precipitate slowly loses weight during ignition, owing to decomposition of the calcium acetate. Pure calcium fluoride remained unchanged on ignition at 800° C. for two hours. Loss of hydrofluoric acid is liable to occur in Rose's method by the action of the acetic acid during the removal of the calcium carbonate from the calcium fluoride. Method (b) suffers from the disadvantage that calcium fluoride becomes contaminated, during the precipitation, with calcium carbonate formed by the boiling ammoniacal solution absorbing carbon dioxide.

The solubility in water of (a) precipitated calcium fluoride dried at 100° C. is 18.3 mgrms. at 18° C., and that of (b) calcined calcium fluoride is 15.1 mgrms. (the volume of water used, not stated, is presumably 1 litre). The values for the solubility of calcium fluoride (not calcined) in various solutions (in mgrms. per litre, temperature not stated) are as follows:—In acetic acid, 30.8 (0.083 N), 38.3 (0.166 N), 40.7 (0.333 N), 49.8 (0.833 N), 58.6 (1.66 N); in ammonium chloride, 20.8 (0.25 N), 25.8 (0.5 N), 27.8 (N), 27.8 (1.66 N); in ammonium acetate, 20.3 (0.333 N), 21.9 (0.71 N), 34.5 (1.42 N), 25.5 (1.66 N); in ammonia, 17.6 (N), 17.5 (1.66 N).

S. G. C.

## Microchemical.

**Micro-crystallographic Identification of Barbituric Alkaloids. G. Denigès.** (*Mikrochem.*, 1931, 9, 316–323.)—The microscopic appearance of the crystals of veronal, soneryl, rutonal, luminal, dial, allyl-*iso*-propyl malonylurate, and allonal are described with the aid of drawings. A fraction of a milligram of the alkaloid is taken for the tests, and is dissolved on a slide in a small drop of ammonia, and then precipitated by a drop of dilute sulphuric acid (1:10 by volume). The crystals are examined with a magnification of about 60 diameters; those of veronal consist of rectangular plates, isolated or in groups, and those of soneryl consist of long needles generally radiating from a centre. Rutonal has characteristic isolated, regular hexagonal plates, or groups of crystals, with the appearance of foliage. Luminal gives crystals in the form of rosettes. Dial gives hexagonal plates, often with sides of irregular length, and groups of rhomboids. Allyl-*iso*-propyl malonylurate gives elongated hexagonal plates, or small rhomboids, and allonal, which is a compound of the preceding alkaloid with pyramidon, is identified by the formation of the allyl-*iso*-propyl malonylurate crystals, whilst the pyramidon part of the molecule is identified by the crystals formed with picric acid, which are yellow elongated prisms, radiating more or less regularly from a centre. The only two crystal formations which might be confused are those of dial and rutonal. These are differentiated by adding a drop of a 2 per cent. solution of sulphuric acid, and

heating gently on a slide. The phenyl substituted alkaloids, such as rutonal and luminal, give an intense brown coloration and yield a precipitate on dilution with cold water. The non-phenyl group gives a slight red-brown colour, with no formation of a precipitate on adding cold water.

J. W. B.

**Determination of Solubility Number: Micro Method for Measuring the Extent to which a Cellulosic Material has been Chemically Modified or Degraded.** C. R. Nodder. (*J. Text. Inst.*, 1931, 22, T416, T424.)—For detecting chemical attack of cellulosic materials, the cuprammonium viscosity method is considerably more sensitive and reliable than the solubility number method in the initial stages of the attack, that is, when the solubility number is less than about 5 for linen goods (log. of the viscosity of 2 per cent. solution less than 1) or less than 2 or 3 for cotton goods (log. viscosity less than about 0.5). For general purposes, however, especially with linen goods, the solubility number method is highly suitable and is decidedly the more simple. The method now described, using 0.1 gm. (or less, if necessary) of material, has been employed for some years as a routine test for the examination of defective materials, for control purposes at the bleach-green, and for purposes of general research.

The material is first boiled for 6 hours in 2 per cent. sodium hydroxide solution under a reflux condenser, in order to remove non-cellulosic impurities and cellulose degradation products soluble in the dilute alkali. It is next washed free from alkali, carefully dried, and cut into fine shreds. With a cloth, Birtwell, Clibbens and Geake's method is satisfactory (*ANALYST*, 1928, 53, 672). Thus, it is often convenient to cut about 10 strips of the cloth,  $1 \times \frac{1}{4}$  inch, parallel to the warp or weft, and to cut, from these, very thin shreds (not more than 1 mm. wide) at an angle of  $45^\circ$  to warp and weft. The shreds are then broken down to a powder between finger and thumb. With yarn, short lengths (less than 1 mm.) are cut from the ends of a small bundle. The air-dry powder (exactly 0.1 gm.) is weighed into a test-tube (conveniently  $5 \times \frac{1}{8}$  inch), having a ground-in glass stopper. The powder is tapped into the bottom of the tube and 1 c.c. of 10 *N* sodium hydroxide is added in drops distributed as evenly as possible; uniform wetting may be promoted by tapping the tube smartly with the finger-tips, but, if wetting is difficult, stirring with a thin glass rod is advisable. As soon as possible the tube is immersed in a water-bath at  $15^\circ$  C. (vacuum flasks are satisfactory), and after 15 minutes 4 c.c. of water are added to dilute the alkali to 2 *N* strength. The tube is left at  $15^\circ$  C., with occasional shaking, for the further period of an hour. For filtering the solution a small fritted glass filter (Schott and Co.'s 30aG3), cut down to within 2 mm. of the disc, is used. This filter is attached to the lower end of a 2 c.c. pipette with thick-walled rubber tubing and immersed in the contents of the tube. The pipette is filled by gentle suction from the water-pump, and the filtered solution (2 c.c.) is run into a 100 c.c. flask and treated with 10 c.c. of 0.5 *N* potassium dichromate solution containing 230 c.c. of concentrated sulphuric acid per litre. The flask is closed with a glass-pear stopper and *immersed* for one hour in a vigorously boiling water-bath, being shaken occasionally. The contents of the flask

are then cooled and titrated with 0.1 *N* ferrous ammonium sulphate solution, with potassium ferricyanide as external indicator. If 10 c.c. of the 0.5 *N* acid dichromate solution require *a*, and the contents of the flask *b*, c.c. of 0.1 *N* ferrous ammonium sulphate, the solubility number =  $1.688(a - b)$ . This result may be corrected for the moisture content of the air-dry material, if thought desirable.

If carefully bleached to a full white by normal processes, a linen damask or sheeting has a solubility number of about 6; a value above 8 is highly undesirable as regards resistance to wear, and a value greater than 10 definitely indicates unsatisfactory bleaching. The maximum desirable solubility number for bleached yarns depends on the counts and quality of the yarn, the degree of bleaching, and the nature of its treatment after weaving, as well as on the use for which the material is intended. In general, the normal value for a yarn of 35's leas bleached "three-quarter white" may be taken as 7, although a value not exceeding 4 is obtainable. With less advanced bleaching or finer yarn, a lower solubility number is desirable, whilst with coarser yarn a somewhat higher number is permissible. With bleached damasks the loss in weight in repeated washes, which has proved a reliable guide to the wearing qualities, is proportional roughly to the square root of the solubility number (*S*) or, more nearly, to  $S^{0.53}$ .

In almost every case the test will show if damage or tendering is the result of chemical attack or of some other action, *i.e.* mechanical or that of micro-organisms. With damaged damasks, sheetings, towellings, etc., which have been repeatedly laundered, comparison of the solubility number of the sewing thread in the hem with that of the cloth will usually show whether general chemical damage was caused in bleaching or in laundering. Further, comparison of the solubility number of the general cloth (or warp and weft separately) with that of the selvedge threads (commonly cotton) will often indicate whether the yarn bleacher or the piece bleacher was responsible for the over-bleaching of materials woven from partly bleached yarns and bleached further in the piece. T. H. P.

**The Alkaline Earth Metals. Methods of Qualitative and Quantitative Micro-analysis.** K. Heller and Z. Stary. (*Mikrochem.*, 1931, 9, 451-520.)—A description with full references of recently developed micro-methods of determination of the alkaline earths. Spectroscopic, colorimetric, nephelometric, and electrometric methods, as well as gravimetric and volumetric methods, are given. The qualitative methods include a number of the new "spot" tests. The methods are described chronologically, with more detailed description of the work since 1926. J. W. B.

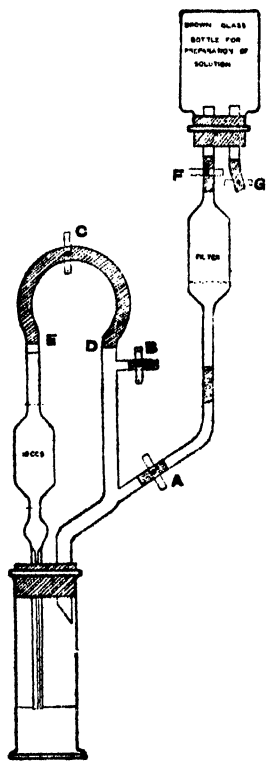
**Detection of Small Quantities of Perchlorates.** D. Krüger and E. Tschirch. (*Z. anal. Chem.*, 1931, 85, 171-176.)—Monnier's methylene blue test for perchlorates (*ANALYST*, 1917, 42, 51) is applied on the micro-scale, and details of the sensitiveness of the test in different conditions are investigated. On the macro-scale, using 1 c.c. of test solution in a test tube, the smallest amount of perchloric ( $\text{ClO}_4$ ) ion detectable is 1000 $\gamma$ , and the limit of dilution 1 : 1000. For the micro-test one drop of the solution under examination is evaporated to dryness

on a slide, and one drop of a 0.2 per cent. aqueous solution of methylene blue is added. Blue and blue violet, needle-shaped crystals and crystal aggregates are formed in the presence of perchlorates, and may be seen under the microscope. The smallest amount recognisable is  $3\gamma$  of  $\text{ClO}_4^-$ , and the limit of dilution is 1:17000; sulphate and acetate ions do not interfere. The test is more sensitive in a saturated solution of sodium acetate, when 0.5 $\gamma$  of  $\text{ClO}_4^-$  is detectable in a 1:100,000 dilution. In the presence of cellulose acetate 2.5 $\gamma$  of  $\text{ClO}_4^-$  is detectable in a dilution of 1:20,000. The test has been applied in an investigation of the use of perchloric acid as a catalyst in acetylations of cellulose. When sulphuric acid is used as a catalyst the cellulose acetate contains some sulphate in the form of ester, but when perchloric acid is used the perchlorate contamination in the cellulose acetate is undetectable by the methylene blue method, and is therefore less than 0.01 per cent.

J. W. B.

## Physical Methods, Apparatus, etc.

**Measurement of the Viscosity of Solutions of Cellulose in Cuprammonium Hydroxide Solution: A Capillary Tube Viscometer.** R. W. Kinkead. (*J. Text. Inst.*, 1931, 22, T411, T415.)—For measuring accurately the viscosity of solutions of cellulose materials in cuprammonium hydroxide solution, the falling sphere method proves satisfactory when the viscosity is relatively high, i.e. when the logarithm of the viscosity of a 2 per cent. solution is above 0.5. With lower viscosities, however, none of the methods previously described gives reliable results. In such cases, the author uses the capillary tube viscometer shown in the figure.



The reservoir is a specimen jar of 12 cm. height, 3 cm. diameter and 90 c.c. capacity, and approximately 50 c.c. of solution are used. The bore of the side tube, D, is 0.7 cm., and the capillary has a bore of 1 mm. and is 10 cm. long below the lower bulb. The upper tube is 7 cm. long above the bulb and its diameter is 0.5 cm. The main bulb has the capacity 10 c.c. between the marks, and the lower bulb, of approximately 1.5 c.c. capacity, assists in preserving the head of liquid. All rubber joints are of pressure tubing. To facilitate emptying and filling, the side-tube is made as wide as possible, and its end below the rubber stopper is cut off at an angle. The volume of the reservoir and side-tube should be such that, when the viscometer is inverted, there is more than sufficient liquid present to fill the bulb tube and the

side-tube. The same reservoir is always used with any one bulb in order to avoid errors due to differences in diameter.

The viscometer is connected at B with a supply of hydrogen and a high vacuum exhaust pipe. The vessel containing the solution of cellulose material in the cuprammonium solution is joined to the reservoir at A, a fritted glass filter of the coarsest grade obtainable being interposed if necessary. The hydrogen supply and pump are also connected at G with the solution vessel, which has all air replaced by hydrogen during the preparation of the solution. The viscometer and filter are exhausted, washed twice with hydrogen through B to remove all air, and again exhausted, screw clip B being then closed. Clip F on the solution vessel is opened and the viscometer reservoir filled through A, the solution being washed through with hydrogen by opening clip G, so that the space in the viscometer above the liquid is filled with hydrogen. Clip A is then closed, and the viscometer disconnected at A and B and inverted. Pressure on the rubber tube, ED, forces the hydrogen from the side-tube, which fills with the solution on releasing the pressure, the liquid flowing up the bulb tube and filling the capillary and bulb. Clip C is then closed and the viscometer is placed upright in a thermostat. Pressure on the rubber tube between C and D removes all the liquid from the side tube up to the clip. Clip C is now opened and the solution allowed to flow through the capillary until the level is slightly below E. When the solution has assumed the standard temperature the capillary is adjusted so that its lower end is just below the surface of the liquid in the reservoir. Any gas bubble trapped under the end of the capillary is removed by applying slight pressure to the rubber tube CD. When clip C is opened the solution flows through the capillary against the back pressure of the rising level in the reservoir and is timed between the marks.

Inversion and filling of the bulb may be repeated as many times as is desired. Repeated observations of the time of flow do not differ by more than 1 or 2 seconds in a flow of 60 to 200 seconds. The filling of the bulb and capillary occupies less than a minute, and the drop in temperature due to removal from the thermostat is very slight, but the viscometer is left in the thermostat for at least five minutes before making a second determination. The instrument may be calibrated by means of a solution containing 40 grms. of cane sugar per 100 grms. of solution.

T. H. P.

**Report on Tintometer Standardisation.** W. D. Hutchins. (*Oil and Fat Ind.*, 1931, 8, 303-304.)—The Colour Committee of the American Oil Chemists' Society makes the following recommendations concerning the standardisation of apparatus and procedure for determining the colour of oils. The tintometer shall be a light-proof metal box with dull black interior. Lovibond red and yellow glasses are to be used, the minimum standard set to consist of: Red, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 7.0, 7.6, 8.0, 9.0, 10.0, 11.0, 12.0, 16.0, and 20.0; yellow, 1.0, 2.0, 3.0, 5.0, 10.0, 15.0, 20.0, and 35.0. For examining maize (corn) and soya bean oils, yellow 30 and red 70 glasses are also required. The red glasses are to be standardised by the U.S. Bureau of Standards. The colour tubes shall have flat, smooth, polished bases of clear colourless glass, and the dimensions: Length 154 mm. over-all, inside diameter, 19 mm.; the tubes are to be marked to show an oil column of 133 mm.

One of the tubes is to be filled, to a depth of 133 mm., with the clear transparent oil at 20° to 24° C., previously filtered, if necessary, through good, heavy grade, close-textured filter paper at 20° to 24° C.; filtration should be noted on the report made. If the oil or fat is not completely liquid at 20° C., it should be liquefied by heating and the colour should be read at a temperature exceeding that of complete liquefaction by not more than 10° C. In matching the colour of the oil with the coloured glasses, use is to be made of only one yellow glass, not more than two red glasses up to and including 13 red, and not more than three red glasses above 13. When only two coloured glasses are used, a colourless glass also must be inserted.

T. H. P.

**Determination of the Hydrolytic Acidity of Decolorising Earths: New Means of ascertaining their Decolorising Power.** H. Utermöhlen. (*Chem. Ztg.*, 1931, 55, 625-626.)—Determination of the hydrolytic acidity of a number of active decolorising earths of German, English, and American origins by the method commonly adopted with soils shows that this acidity increases with the decolorising effect of the earths on a sample of soya bean oil, previously treated with dilute sodium hydroxide solution. To determine the hydrolytic acidity, 100 grms. of the earth are mechanically shaken for an hour with 250 c.c. of *N* sodium acetate solution in a Stohmann 500 c.c. flask. After filtration, 125 c.c. of the liquid are titrated with 0.1 *N* sodium hydroxide solution in presence of phenolphthalein. The number of c.c. of the alkali used represents the hydrolytic acidity. If a result is required quickly, the earth and acetate solution may be shaken together by hand for a few minutes, after which time the reaction is nearly complete. It should be borne in mind that the preparation of highly active decolorising earths involves treatment with acid, small proportions of which are always retained, even after thorough washing. The value found for the hydrolytic acidity must be corrected by subtraction of the corresponding value for this acid. Many earths exhibit such marked absorptive properties that insufficient filtrate is obtained; in such cases 75 c.c. are titrated and the number of c.c. required is multiplied by 2. Further, with highly active earths, the titration is best carried out with *N* alkali.

T. H. P.

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## Reviews.

**SOLVENTS.** By T. H. DURRANS, D.Sc., F.I.C. Pp. xv+180. Revised Second Edition. London: Chapman & Hall, Ltd. 1931. 10s. 6d.

The first edition of this book, extremely useful within its restricted scope, was welcomed by us in November last (1930, 50, 726). We can welcome this second edition, with its extra 36 pages, and with the satisfaction of knowing that its appreciation by chemists and manufacturers has resulted in so practical an expression.

The book still discusses solvents primarily from the point of view of their utility in the manufacture or use of cellulose esters. There is thus little or no mention of their value as extractors of drugs and in the preparation of tinctures, as fat extractors in the food and allied industries, as rubber solvents and in the rayon industry, as dry-cleaning agents, or of their multifarious applications to more purely chemical operations, such as crystallisation, in works and laboratory.

We could not really cavil at these omissions, did not the very general title suggest the book to have a wider scope than is the case.

Although Part II of the book, dealing with specific solvents, is largely a compilation from tables of constants and manufacturers' lists, it should be very convenient to many technicians as well as to lay heads of departments. It is, however, to be regretted that certain commercial products have not been analysed, so that statements like "'Palatinol A' and 'Anozol' are reputed to consist of di-ethyl phthalate" might have been replaced by exact information.

Part I consists of a series of general, and fairly elementary, chapters on the chemical and physical properties and influence of solvents—solvent action, solvent power, plasticising action, solvent balance, viscosity, vapour pressure, inflammability and toxicity. In Part II organic solvents are treated individually under their respective main groups.

The production of the book is excellent, and the publishers are to be congratulated and thanked for having added 36 pages to it without increasing the price.

A. L. BACHARACH.

**RECENT ADVANCES IN MICROSCOPY. BIOLOGICAL APPLICATIONS.** Edited by A. PINEY, M.D., with contributions by five authors. Pp. vii+260, with 83 illustrations. London: J. & A. Churchill. 1931. Price 12s. 6d.

Owing to the ever-increasing specialisation necessary in science at the present time it would be difficult for any one reviewer to deal fully with the whole of the



contents of this work, since it treats of results obtained in the more remote and limited departments of microscopy.

The volume is intended to give a general idea of the more recent developments in the applications of the microscope to biological structure and phenomena, and whilst of considerable value to the microscopist and biologist, from the nature of the subjects dealt with would probably prove difficult reading for the layman.

The first section of the book is contributed by the editor, and after a brief but useful introduction is devoted to general discussions of the cytology of various animal tissues and glands. In the "Microscopy of the Living Eye," by B. Graves, an excellent description is given of the use of the recently developed slit lamp in conjunction with the microscope for the detection and observation of abnormal conditions and pathological changes in various parts of the human eye. The methods of illumination illustrated in this section not only demonstrate in an able manner the use of the instrument to the oculist, but in addition would also provide excellent test exercises for advanced students of optics or microscopy. The remaining two sections, by E. W. MacBride, H. R. Hewer and E. C. Barton-Wright, comprise about two-thirds of the volume and are devoted to the study of animal and vegetable cytology and illustrate the remarkable developments taking place in the minute study of the simple cell. Not only are the results of a number of investigations in this exacting branch of science given, but able and erudite comments, criticisms and comparisons are made by the authors on the conclusions reached by the various workers. From these sections one gains an insight into the vacuome, nucleus, mitochondria, Golgi apparatus and other internal structures of the living cell in various species of animals and plants, together with information on the development of ova, spermatozoa, spores and the chromosomes which play so great a part in the study of genetics. The whole of the text is well written and serves as an admirable summary of the progress being made in subjects involving difficult technique, the results of which are at present in many cases in the early controversial stage, but which point the way to further developments which cannot fail to become of value. The authors have succeeded in converting into a coherent and lucid form much matter which might otherwise have appeared decidedly unattractive, but the value in this respect would have been much enhanced by the provision of a comprehensive glossary, since many of the newer terms used are known to relatively few, and are not to be found in any dictionary. The book is generously illustrated with excellent diagrams, and the principal sections are provided with bibliographies. The text appears to be free from errors of any kind, and the index is accurate and reasonably complete. To those interested in the subjects dealt with the volume will prove exceedingly good value for the price charged.

T. J. WARD.

A STUDY OF HAIRS AND WOOLS. By JOHN GLAISTER, Junr., D.Sc., M.D. Pp. 172, with 35 plates. Cairo: University Press. 1931. Price 40s.

This elaborate work originates from the Faculty of Medicine of the Egyptian

University, and has been published with the financial assistance of the Egyptian Government. Its scope is confined to the hairs and wools of the mammalia, considered from the medico-legal aspect, and its aim has been to provide a reference atlas by means of which hairs of unknown animals can be recognised and distinguished from those of man.

In his introduction Professor Glaister rightly lays stress upon the point that a photographic record is essential for the legal demonstration of the identity of a hair, but against this must be set the fact that the distinctive features of a hair can often be brought out more clearly by means of a camera lucida drawing than by photo-micrography, and that, subject to the permission of the Court, a photo-micrograph may well be supplemented by a diagram. Useful directions for preparing cross-sections of hairs are given, and the photographic methods used are described in detail.

The photo-micrographs of the hairs of the various animals have been arranged in accordance with the zoological classification of Flower and Lydekker, and valuable explanatory details of the characteristics of each order elucidate the plates; it is safe to assert that never before has such a complete collection of reproductions of the hairs of mammalia, ranging from the duck-bill platypus to man, been made available, and the author may be congratulated on having completed such a laborious task. Although some of the animals whose hairs are photographed and described are unlikely ever to form the subject of a criminal investigation, yet there is justification for including them in a medico-legal atlas of hairs and wools, since a witness might well be asked in cross-examination whether the hair attributed to one animal could not possibly have come from another. The series of plates of human hair represent photomicrographs of hairs at various ages and from different parts of the body, but the reproductions are somewhat disappointing, as many of them fail to bring out the distinctive structure of the cortex. There is also no account of the racial differences to be found in certain human hairs, such as those of the Australian aborigines, or of characteristic affections of human hair, such as ringed hair, and so on.

From what has been said it will be gathered that this atlas will sometimes be found helpful by those who have to make microscopical examinations of hairs and fibres, but that each investigator should supplement it by his own photo-micrographs and drawings. The absence of an alphabetical index (as distinct from the list of the contents of the plates) is a drawback.

EDITOR.

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## Publications Received.

- RECENT ADVANCES IN ORGANIC CHEMISTRY.** By A. W. STEWART, D.Sc. Vols. I and II. London: Longmans, Green & Co. Price 21s. net each Vol.
- PERKIN AND KIPPING'S ORGANIC CHEMISTRY.** Part I. Entirely new edition. By F. S. KIPPING, Sc.D., F.R.S., and F. B. KIPPING, M.A., Ph.D. London: W. & R. Chambers, Ltd.
- FORENSIC CHEMISTRY AND SCIENTIFIC CRIMINAL INVESTIGATION.** By A. LUCAS, O.B.E., F.I.C. 2nd Edition. London: Edward Arnold & Co. Price 18s. net.
- THE SCIENTIFIC DETECTIVE AND THE EXPERT WITNESS.** By C. AINSWORTH MITCHELL, D.Sc. 2nd Edition. Cambridge: W. Heffer & Sons Ltd. Price 3s. 6d. net.
- QUANTITATIVE CHEMICAL ANALYSES.** By CLOWES and COLEMAN. Revised by D. STOCKDALE, Ph.D., and J. DEXTER, B.Sc. 13th Edition. London: J. & A. Churchill. Price 18s.
- RECENT ADVANCES IN PHYSICAL CHEMISTRY.** By S. GLASSTONE, D.Sc., F.I.C. London: J. & A. Churchill. Price 15s.
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- ORGANIC SYNTHESSES.** Editor in chief: C. S. MARVEL. Vol. XI. London: Chapman & Hall. Price 8s. 6d. net.
- LES EAUX D'EGYPTE.** By A. AZADIAN. Vols. XII and XIII. Cairo: Imprimerie Nationale.
- STUDIES IN VITAMIN B<sub>1</sub>: CONCENTRATION FROM RICE POLISHINGS.** By I. A. SIMPSON. Bull. No. 2, The Institute for Medical Research, Federated Malay States.
- A BRIEF INTRODUCTION TO THE USE OF BEILSTEIN'S HANDBUCH DER ORGANISCHEN CHEMIE.** By E. M. HUNTRESS, Ph.D. London: Chapman & Hall. Price 5s. net.
- DIE ARBEITSMETHODEN DER TRINKWASSER BIOLOGIE.** By H. BEGER. (Abderhalden, Handbuch der biologischen Arbeitsmethoden, Lieferung 366.) Berlin: Urban & Schwarzenberg. Price RM.5.50.

# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, October 7th, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—Charles Hubert Francis Fuller, B.Sc., F.I.C., Ganesh Chandra Moitra, B.Sc., Eric Charles Wood, B.Sc., A.R.C.S., Robinson Pearson Wood, M.Sc., F.I.C.

Certificates were read for the second time in favour of:—Raymond Merefield Edwards, B.Sc., Llewelyn John Howells, B.Sc., Donald Neil McArthur, D.Sc., Ph.D., F.I.C., F.R.S.E., James Sword, M.A., B.Sc., Ph.D., A.I.C.

The following were elected Members of the Society:—George Brown, A.I.C., Charles Loudon, B.Sc., A.I.C., Charles Percy Money, B.Sc., F.I.C., Martin Priest, F.I.C., Arthur Goodyear Simpson, M.A., Gerrish Smith.

The following papers were read and discussed:—"The Identification of Wood and Wood Charcoal Fragments," by J. Cecil Maby, B.Sc.; "The Examination of Dyed Leather in Cases of Alleged Dermatitis," by T. Callan, M.Sc., Ph.D., F.I.C., and N. Strafford, M.Sc., F.I.C.; and "The Determination of Chlorides in Dairy Products and Biological Material," by W. L. Davies, M.Sc., Ph.D., F.I.C.

### NORTH OF ENGLAND SECTION.

A MEETING of the Section was held in Manchester on October 10th, 1931. The Chairman (Mr. C. J. H. Stock) presided, and there was an attendance of thirty-five, including the President (Dr. Dunn).

An address was given by Prof. W. H. Roberts, M.Sc., F.I.C., on "The Analyst in the Witness-Box." A discussion followed in which many of the members took part.

In the morning, the members, by kind invitation, visited the premises of Messrs. Allied Dairies, Ltd., and inspected their plant. Lt.-Col. J. W. Brittlebank, C.M.G., on behalf of the firm, entertained the visitors to lunch.

## Obituary.

### HENRY DROOP RICHMOND.

HENRY DROOP RICHMOND was born at Hampstead on January 17th, 1867, and studied chemistry under Temple Orme at University College School. He afterwards proceeded to Finsbury Technical College, and later became one of four or five assistants in the laboratory of Otto Hehner. It was in this laboratory, about 40 years ago, that I first met Richmond. He had a marked faculty for helping the younger members of the staff, and never failed to give good advice. He was always recognised as a sound mathematician, and was the first to apply modern statistical methods to problems connected with milk analysis.

After leaving Hehner's laboratory, he joined the staff of the Khedival Laboratory at Cairo as second chemist, relinquishing this position in 1892.

On returning to England, he was appointed Chief Chemist to the Aylesbury Dairy Company, in succession to Vieth, whose admirable work he continued and developed. He remained with this firm for some 23 years, and there enhanced his reputation as a chemist of outstanding ability. While holding this appointment, he wrote his well-known standard book on Dairy Chemistry, invented the Milk Slide Rule, and contributed a large number of papers to *THE ANALYST* on many subjects connected with milk. His extensive experience in the analysis of milk and the statistical treatment of milk records proved to be of great value to the Departmental Committee on Milk and Cream Regulations in 1901.

Droop Richmond was a valued member of several scientific societies. He became a Fellow of the Institute of Chemistry in 1887, and served on its Council on two occasions. He was also one of the older members of the Society of Public Analysts, having joined in 1890, acted as Treasurer in 1910-12, and as Vice-President in 1909 and 1914-15, and he was on the Council during 1893-96, 1900-1, 1907-8. In addition to this, he served as Vice-Chairman of the Nottingham Section of the Society of Chemical Industry.

Richmond went to Messrs. Boots Pure Drug Company at Nottingham in 1915, primarily to work on the production of casein foods and of glycerophosphates. Shortly afterwards he was appointed Chief Analyst, and occupied that position until his death, although for the past two years his health had compelled him to abandon active work.

While with this company he published numerous papers in *THE ANALYST* and elsewhere, dealing with the analysis of drugs, some of the more important being his study of the selective hydrolysis of saccharin in the presence of its associated impurities (published in 1919), which led to a more exact method for the valuation of saccharin, and his paper on the action of hygroscopic drugs (potassium carbonate, etc.) on the glass containers in which they are stored, leading to the contamination of the drug with arsenic and lead (*ANALYST*, 1923, 48, 260.)

His expressed belief in the value of physical methods of analysis (*ANALYST*, 1903, 28, 141) was maintained in his series of papers, commenced before his period

of work at Nottingham and continued up to 1919, entitled "Studies in Steam Distillation," and in a number of other papers involving the use of physical methods, most of which were published in THE ANALYST.

As a further example of Richmond's many-sided activities, reference may be made to his communication to the Journal of the Chemical Society (1892, 56, 491) on the supposed discovery of a new element in an Egyptian mineral. The reactions of the substance isolated were described, and its atomic weight was calculated to be 228.

His interest in the younger members of his laboratory staff at Nottingham was shown in many ways. He conducted classes for them in Latin and higher mathematics, took part in week-end walks with them, and, while his health allowed, joined them in both outdoor and indoor sports. His interest in education extended further than his own department, and for some time he conducted a "Laboratory Corner" in the firm's magazine, for the benefit of pharmaceutical students in the firm's retail branches.

After a severe operation in 1924, he never really recovered, and, although he attempted to struggle on, physical weakness compelled him to abandon active work early in 1930.

Those who worked with him during the last few years bear witness to his fortitude and patience, and his sheer determination to carry on his work in spite of growing pain and weakness, will always remain as a great trait of a kindly and lovable character.

I would like to bear testimony to the valuable help he so ungrudgingly gave me during a friendship which lasted over 40 years.

LEONARD BOSELEY.

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## Death.

We greatly regret to have to record the death, on September 18th, of Dr. CHARLES A. KEANE, who was Vice-President of the Society in 1921-1922.

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## Standards for Jam.

### PERCENTAGE OF SOLUBLE SOLIDS.

As the determination of total soluble solids set out in the Final Memorandum "by refractometer reading when cold" occasions small inaccuracies, the Council has decided that the refractometer reading shall be taken at a temperature of 20° C.

The appropriate Committee of the Food Manufacturers' Federation, Incorporated, has agreed to this alteration.

## Evaluation of the Menthone Content of Peppermint Oil.

By J. REILLY, D.Sc., N. NOONAN, M.Sc., AND P. J. DRUMM, Ph.D.

(Read at the Meeting, November 4, 1931.)

EXPERIMENTS on the possibility of the economic production of Irish oils of lavender, peppermint, and dill have engaged attention in this laboratory for a number of years (*Econ. Proc. Roy. Dub. Soc.*, 1926, 2, Nos. 16, 17, 18, 19). The determination of the carvone and menthone content of dill and peppermint oils, respectively, presented some difficulty, inasmuch as the methods already in use did not always give concordant results.

Power and Kleber's method of determining menthone (*Pharm. Rundschau*, 1894, 12, 162) consists in the determination of the menthol content of the oil before and after reduction. The menthol content is first determined by acetylation of the oil, followed by saponification. The oil is next reduced in alcoholic solution by means of sodium. The reduced oil is isolated, then acetylated, and the acetylated oil is afterwards saponified. From the increase in the menthol content the amount of menthone is determined. This method is tedious and leads to different values for the menthone content, according to the exact procedure.

A method in which carvone is determined as its semicarbazone was evolved by us, and has already been communicated to this Society (*ANALYST*, 1928, 53, 209). In the present paper this method has been extended, with satisfactory results, to the determination of the menthone content of peppermint oil.

The pure menthone for preliminary experiments was prepared from the commercial product. This was converted into its semicarbazone by warming an aqueous alcoholic solution of the menthone semicarbazide hydrochloride (Kahlbaum) and sodium acetate. The precipitated *l*-menthone semicarbazone was collected and recrystallised from alcohol until it had a constant melting point (185° C.). The rotation of the menthone regenerated from its semicarbazone by hydrolysis with dilute acids was found to be considerably below that recorded in the literature for pure *l*-menthone. This is possibly due to the racemising action of the acid. In an attempt to avoid this, the method of Kon (*J. Chem. Soc.*, 1930, 1616) for the purification of sensitive ketones was employed. This consists in mechanically shaking the semicarbazone, suspended in petroleum spirit to remove the ketone from the sphere of reaction, with the calculated amount of  $N/2$  sulphuric acid until hydrolysis is complete. This method was abandoned, as it was found that, under the conditions stated, very little hydrolysis of menthone semicarbazone took place.

Ultimately it was found that hydrolysis of the semicarbazone with the theoretical amount of  $N/2$  sulphuric acid and removal of the menthone in a current of steam as it was formed, gave a menthone of fairly satisfactory rotation.

**DETERMINATION OF PURE MENTHONE.**—Two grms. of menthone were added to a solution of 3·4 grms. of semicarbazide hydrochloride and 3 grms. of fused sodium acetate in 45 c.c. of water. Forty-five c.c. of 93 per cent. alcohol were added to the mixture until a clear solution was obtained, and the whole was gently warmed on a water-bath for a few minutes. Separation of the semicarbazone occurred almost immediately. The whole was allowed to remain at room temperature over-night. The precipitated semicarbazone was collected, dried and weighed. The following results were obtained:—

Menthone taken. Grms.	Semicarbazone precipitated. Grms.	Semicarbazone in solution.	Total semicarbazone. Grms.	Menthone estimated. Grms.
2	2·49	{ 45 c.c. water 45 c.c. alcohol 0·10 gm. }	2·59	1·890
5	6·28	{ 112·5 c.c. water 112·5 c.c. alcohol 0·25 gm. }	6·53	4·765

In a series of determinations the amounts found were all approximately 95 per cent. of those taken, and a factor might be used to allow for the deficiency.

**DETERMINATION OF MENTHONE IN PEPPERMINT OIL.**—The method of procedure is the same as for pure menthone. Ten grms. of peppermint oil were added to a solution of 3·4 grms. of semicarbazide hydrochloride and 3 grms. of fused sodium acetate in 30 c.c. of water. Sixty c.c. of 93 per cent. alcohol were added to the mixture until a clear solution was obtained. The solution was then gently warmed on the water-bath, when precipitation of the semicarbazone commenced almost immediately. The whole was allowed to stand over-night, when most of the semicarbazone had separated in crystalline form. This was collected, dried and weighed. The crude semicarbazone in all the test determinations melted at 184–185° C. The solubility of menthone semicarbazone in a mixture of 30 c.c. of water and 60 c.c. of alcohol was 0·16 gm.

Several determinations with Irish peppermint oil gave closely agreeing results, the extreme figures being shown in the following table. No allowance is made for a factor.

Irish peppermint oil taken. Grms.	Semicarbazone precipitated. Grms.	Semicarbazone in solution (alcohol, 60 c.c.; water, 30 c.c.). Grms.	Total semicarbazone. Grms.	Percentage of menthone in Irish peppermint oil.
10	2·854	0·16	3·014	22·0
15	4·230	0·16	4·390	21·4

**OXIME TITRATION METHOD.**—In order to obtain comparative figures the menthone content of peppermint oil was also determined by means of hydroxylamine (for the particulars of which method we are greatly indebted to Messrs. Bush & Co., Hackney).



By this method hydroxylamine hydrochloride is allowed to react in the cold with the oil, and the liberated hydrochloric acid is titrated as described below. Each c.c. of  $N/2$  potassium hydroxide solution is equivalent to 0.077 grm. of menthone.

About 10 grms. of peppermint oil are added to 25 c.c. of a solution of  $N/2$  hydroxylamine hydrochloride in 80 per cent. alcohol (which has been previously neutralised, if necessary, with alkali). The solution is allowed to stand for half-an-hour and then back-titrated with  $N/2$  alcoholic potassium hydroxide solution, brom-phenol blue being used as indicator. The figures by this method are about 2 per cent. lower than when the process is carried out with hot solutions.

The following table shows the results obtained with various oils by the above methods:—

Oil.	Percentage of menthone.	
	Semicarbazone method.	Oxime method.
Irish .. .. .	22.00	21.40
American .. .. .	17.25	17.00
English (Messrs. Bush & Co.) .. .. .	18.25	17.45
English (Messrs. Stafford, Allen & Co.)	16.70	17.00

From the above table it is seen that the semicarbazone method, even when no allowance is made for the factor previously indicated, generally gives higher values than the oxime method. The semicarbazone isolated is a pure substance. This method, therefore, gives at least a minimum value for the menthone content of peppermint oil. It would appear, therefore, that the oxime method does not estimate all the menthone present in the oil.

THE CHEMICAL STATION,  
UNIVERSITY COLLEGE, CORK.

#### DISCUSSION.

Mr. C. E. SAGE said that the authors' opening remarks on the possibility of the profitable production of essential oils of lavender, peppermint and dill in Ireland were not meant for criticism by a scientific meeting, but his commercial experience with the preparation of all three oils in Spain and America would suggest that further enquiries should be made there before embarking on the cultivation of the plants in Ireland; English growers could give some useful advice regarding the profits to be derived from peppermint growing in England. The climate of Ireland might have a very considerable effect on lavender, similar to that produced by different altitudes in Spain. The dill, to do any good at all, required sunshine and plenty of it, whilst the peppermint needed something more than chemical, botanical or agricultural experience to make its cultivation profitable.

The determination of menthone by the semicarbazone method might be theoretically possible and scientifically desirable, but in actual practice it was not reliable. His experience had been that the semicarbazone did not always separate immediately, that it did not all separate in 24 hours, and, even after standing three days and filtering, another small crop might be gathered. In comparison with the oxime method, the semicarbazone method was slow, cumbersome and uncertain, and yielded, in his hands, entirely erroneous results. Taking a commercial menthone from a well-known source, the oxime method gave consistent

results, indicating 88 to 89 per cent. purity, whilst by the carbazone method as described by the authors only 70 per cent. was obtained. With an oil known to contain 25 per cent. of menthone, the hydroxylamine method yielded 25.2 and 25.4 per cent., whilst the semicarbazone method yielded only 14.3 per cent. after standing 24 hours, and a further crop of crystals after 48 hours. Another comparative experiment with a mixture containing carvone yielded 55 per cent. by the oxime method, and only 40.5 per cent. by the semicarbazone method, as described by Reilly and Drumm in a paper read before the Society in 1928. Such results indicated the need for further examination of the method before its acceptance as reliable.

The authors' statement that the hydroxylamine method gave lower results than that suggested by them might be accounted for by the fact that they made the test cold, whereas it was known that even at a temperature of 78° C. it took an hour to complete the reaction, and, in the cold, consistent results were not obtained by different workers.

Mr. T. T. COCKING sent the following communication, which was read to the meeting.

The authors' results for the menthone in peppermint oil strike me as being on the low side. I have carried out a number of determinations by the hydroxylamine method, and, with one exception—that of a very old sample of English oil—all oils have contained from 22 to 35 per cent. of menthone. The method I have used is that which has been recommended by C. T. Bennett and myself for the determination of ketones in essential oils, with special reference to carvone in the oils of caraway and dill. The reagent is a normal solution of hydroxylamine hydrochloride in 90 per cent. alcohol adjusted to the full yellow colour of dimethyl yellow, and the reaction is carried out at a temperature of about 70° C., the free acid liberated being titrated intermittently with normal alcoholic potash until the reaction is complete.

The reaction proceeds rapidly at first, about 90 per cent. of the menthone being converted into oxime in the first 20 minutes. After this the reaction slows down, but is usually complete in from 40 to 60 minutes. It was found that when the reaction was carried out in the cold with a half-normal reagent prepared with 80 per cent. alcohol, combination was very slow and did not proceed to completion even after many hours. There is no doubt that the half-hour's contact in the cold allowed by the authors is insufficient, and is responsible for the low results given by this method.

I note that when using the semicarbazone method on peppermint oils, the authors make a correction for the solubility of the semicarbazone in the volume of dilute alcohol used, but do not state whether the solubility is affected by the presence of the non-menthone portion of the peppermint oil.

While the method advocated will, no doubt, be useful for the separation and identification of menthone, it is doubtful if the results will attain the accuracy of the hydroxylamine method when this is carried out under the best conditions.

Dr. REILLY sent the following reply: It has been shown by the staff of the Chemical Department, University College, Cork, in the *Economic Proceedings of the Royal Dublin Society (loc. cit.)* that the production of essential oils from Irish-grown plants is practicable, and that the oils compare favourably with the best Mitcham products. The further development of the industry is mainly a question of finance.

The semicarbazone method is not intended to replace the oxime method, which gives figures of technical value. The menthone semicarbazone, isolated as described, is a single organic compound with a definite melting point, and in

this respect it differs from the oxime reaction product, which may be more complex, especially if the reaction is carried out in a warm solution. With experience, the method gives concordant results, and with synthetic mixtures the menthone content can be determined fairly accurately. It is not claimed that the method can be used with all commercial oils, as it has only been studied to a limited extent.

In carrying out the hydroxylamine method the conditions as to temperature, given in the literature, are generally vague. In the particular method followed, directions to carry out the reaction *in the cold* were definitely given.

Further work is in progress on the application of the semicarbazone method, especially for the separation of pure ketones.

## A New Method for Detecting Decomposition Products in Anaesthetic Chloroform.

By NOEL L. ALLPORT, A.I.C.

(*Read at the Meeting, May 6, 1931.*)

It is a well-established fact that perfectly pure chloroform is liable to oxidation and that one of the products formed is phosgene (carbonyl chloride). This decomposition takes place so readily that all pharmacopoeias direct the addition of a small amount of alcohol which acts as a preservative. According to Clover (*J. Amer. Chem. Soc.*, 1923, **45**, 3133), alcohol behaves as an anti-catalyst, and retards, without entirely preventing, the oxidation. The formation of phosgene is, however, inhibited, since alcohol will at once react with any that is formed, producing mainly hydrochloric acid and ethyl carbonate. Notwithstanding this, it has been customary to examine anaesthetic chloroform for traces of phosgene, which obviously could not be present until all the alcohol had been decomposed and a corresponding amount of hydrochloric acid formed. It is obvious that, in the examination of chloroform for evidence of decomposition, attention should be directed to the detection of the minutest trace of hydrochloric acid, but the methods hitherto available are not as sensitive as could be desired. A new test is here proposed which, it is hoped, may be found to be an advance on existing tests. It is expressly applicable to B.P. chloroform.

Three of the tests at present commonly used are directed specifically to the detection of hydrochloric acid or phosgene. These are the tests with silver nitrate, barium hydroxide, and benzidine.

The silver nitrate method involves shaking the chloroform with water, allowing separation to take place, and examining portions of the aqueous liquid for chlorides and for acidity. This test is included in most of the pharmacopoeias, and is quoted as a limit test for hydrochloric acid. As a rough preliminary examination it undoubtedly serves a very useful purpose, since any chloroform failing to pass the test could not possibly be considered as sufficiently pure for anaesthetic

use. It will be shown, however, that the method is unsuitable for detecting traces of hydrochloric acid.

The barium hydroxide test, originally suggested by Ramsay (*J. Soc. Chem. Ind.*, 1892, 11, 772), was modified by Baskerville and Hamor (*J. Ind. Eng. Chem.*, 1912, 4, 571). It is conducted by introducing 15 c.c. of the sample into a stoppered bottle of 25 c.c. capacity, adding sufficient aqueous barium hydroxide (1 in 19) to fill the bottle, and allowing the mixture to stand, without agitation, for three hours in a dark place. The formation of a film of barium carbonate at the junction of the two layers of liquid is said to indicate the presence of phosgene. Actually, this test responds to carbon dioxide; it is, therefore, unsatisfactory as a test for phosgene. It is also very difficult to operate, owing to the interference caused by the carbon dioxide in the air. Positive reactions are occasionally obtained by this test, owing to traces of carbon dioxide dissolved in the chloroform.

The benzidine method, which was devised by Utz (*Pharm. Zentr.*, 1917, 58, 1), is a distinct improvement upon the foregoing. In its original form it has been included in the German Pharmacopoeia (D.A.B. VI). The benzidine (0.1 grm.) is added to 20 c.c. of chloroform, filling a completely dry, stoppered flask and allowed to stand in the dark for 24 hours. Utz states that 100 parts per million of phosgene will be indicated by a cloudiness, and that ten times this amount produces a yellowish-white precipitate. In the same paper it is pointed out that a turbidity or precipitate is also produced by hydrochloric acid. This test is more delicate when only one-fifth of the amount of benzidine originally prescribed is employed. In this way most of the colour due to the reagent itself is eliminated, but still the reaction is not sufficiently sensitive, and the necessity for standing over-night is a further disadvantage.

While investigating the foregoing process it occurred to me that advantage might be taken of the very delicate colour reactions produced by various phenols with aldehydes in the presence of condensing agents. The well-known test for detecting formaldehyde by the addition of resorcinol in the presence of sulphuric acid is an example of this type of reaction. The Kreis test for rancidity of oils and fats also depends upon the same principle, *viz.* the detection of traces of aldehyde formed prior to the development of free acid. Conversely, the reaction between phenol and aldehyde may be used to detect the presence of a condensing agent. This principle is embodied in the Gunsberg test for the detection of free hydrochloric acid in gastric juice. It consists in mixing an alcoholic solution of phloroglucinol and vanillin with the specimen and evaporating to dryness, a rose-red colour being produced if free hydrochloric acid is present. Panton (*Lancet*, 1918, [ii], 125) has suggested the substitution of either orcinol or resorcinol for phloroglucinol.

Preliminary experiments on these lines soon led to promising results, and it was found that by the adoption of the technique to be described one may detect extremely small amounts of hydrochloric acid in chloroform. The reagents employed are resorcinol and vanillin. The colour reaction is extremely delicate. In

this connection interest attaches to the work of La Wall (*Amer. J. Pharm.*, 1905, **77**, 392), who, when testing for the presence of formaldehyde in vanilla ice cream by the resorcinol test, obtained a positive reaction, although other tests gave negative results. The colour given by the resorcinol was found to be due to the vanillin present and not to formaldehyde, and further investigation showed the resorcinol test to be capable of detecting one part of vanillin in 200,000 of cream. Crocker (*Ind. Eng. Chem.*, 1925, **17**, 1158) has investigated these colour reactions between phenols and aldehydes, and has found that, by using the pure substances, resorcinol gives a colour with a 1 in 2,000,000 dilution of vanillin when mineral acid is present.

In applying the method for the purpose of detecting the formation of phosgene in chloroform it was necessary to prepare specimens of chloroform representing specific degrees of decomposition. This was done by the addition to B.P. chloroform of known quantities of phosgene. For convenience the concentration of decomposition products has been expressed in terms of phosgene.

**EXPERIMENTAL.**—An approximately 10 per cent. (w/v) solution of phosgene in B.P. chloroform was prepared by passing the gas into the solvent from a cylinder. The strength was determined by adding a known volume of the solution to a relatively large bulk of water contained in a stoppered flask and titrating the hydrochloric acid produced after agitating the mixture at frequent intervals during a period of about three hours. From this solution dilutions of the desired strength were made, the purest anaesthetic chloroform being used.

Preliminary experiments showed that if a solution of resorcinol and vanillin in B.P. chloroform was mixed with a moderately strong chloroformic solution of phosgene, a red precipitate formed more or less quickly according to the concentration of the reacting substances. This red substance probably belongs to the triphenylmethane group of dyes, and is only very slightly soluble in chloroform. A series of tests was made in which stoppered bottles, each holding 15 c.c. when completely filled, were used. Fifty mgrms. each of resorcinol and vanillin were added to each bottle, and the bottles were then completely filled with chloroformic solutions of phosgene, the dilutions ranging from one to twenty parts per million. After being securely stoppered the bottles were placed in a darkened cupboard. At the same time parallel experiments were made by the silver nitrate test and by the modified benzidine method. Blank tests with purest anaesthetic chloroform were made in each case. After 24 hours a slight turbidity was shown by the benzidine method in chloroform containing 5 parts per million. Silver nitrate revealed a slight opalescence when 10 parts per million or more were present. The resorcinol and vanillin test showed a distinct turbidity with 5 parts per million, and a faint but definite turbidity with 2 parts per million. Stronger solutions of phosgene showed pink precipitates in the latter test. When these reacting liquids are shaken with dilute aqueous alkali solutions the precipitate is dissolved in the alkaline layers, forming red solutions and thereby rendering the test much more delicate. By this method a rich pink aqueous layer is obtained with chloroform

containing 1 part per million of phosgene, whilst a blank test shows no pink coloration whatsoever, the aqueous layer remaining colourless for about two minutes, after which it begins to darken by reason of the resorcinol present. In subsequent experiments it was found that by reducing the quantity of reagent used to 20 mgrms. each of resorcinol and vanillin this tendency to darkening is decreased.

A series of tests was conducted in an endeavour to limit the time of standing. After remaining in a darkened cupboard for one hour the contents of the bottles were transferred to suitable tubes and immediately shaken with 5 c.c. of 1 per cent. ammonia solution. Although no coloration or turbidity was visible in the chloroform prior to the addition of ammonia, yet with it distinct pink aqueous layers were obtained, even with chloroform to which only 1 part per million of phosgene had been added, whilst pure chloroform yielded a colourless alkaline layer. The depth of colour was clearly proportional to the amount of phosgene present. With periods of shorter duration than one hour the results were not found to be so satisfactory.

Further experiments were made to ascertain the effect of light. With concentrations of phosgene of 5 parts per million and over, the pink colour developed in the chloroform itself after exposure to diffused daylight for one hour. Comparisons made with and without exposure to daylight showed that there was no apparent difference in the colour of the alkaline layer for equal concentrations of phosgene. It is recommended that the test mixtures should stand in darkness, since more uniform conditions are thus obtained. It was also shown that the exclusion of air by the use of completely filled bottles is immaterial to the success of the test. In consequence, transferring the chloroform to a larger vessel, prior to adding the ammonia, is unnecessary. The limit of sensitiveness for the resorcinol-vanillin test carried out as herein described is about one part of phosgene in two million parts of chloroform.

It is interesting to note that after the test mixtures have stood for about 15 minutes the aqueous layers have darkened in all cases, but, in the complete absence of free acid the chloroform is quite clear, and is turbid when a positive reaction has been obtained. Even when the equivalent of only 1 part per million of phosgene is present, the turbidity is quite obvious, and it becomes more marked with increasing proportions of impurity.

Besides resorcinol and vanillin many other phenols and aldehydes were tried in various combinations, but in no case was the result as satisfactory. Resorcinol and piperonal yielded a pink coloration or precipitate in the chloroform, varying with the concentration of phosgene employed, but the sensitiveness was much less than in the resorcinol and vanillin test. The substitution of phloroglucinol for resorcinol in the adopted test was ineffective.

The behaviour in this test of alcohol-free chloroform to which phosgene had been added was then examined, and the results showed the presence of alcohol to be necessary. Absolute chloroform was prepared by washing B.P. chloroform repeatedly with water, drying over anhydrous potassium carbonate and then

twice distilling, the first and last runnings being rejected. Dry phosgene was passed into a portion of this absolute chloroform and, after titration as before described, dilutions were made with the remainder of the same solvent.

Even when 0.1 per cent. of phosgene was present the resorcinol and vanillin test showed only a faint pink coloration in the chloroform. On adding about 1 per cent. of absolute alcohol, however, a red precipitate formed in a few minutes. On the other hand, the response to the benzidine test was better in the absence of alcohol. The turbidity was produced at once when only 5 parts per million of phosgene were present; whereas with B.P. chloroform and the same concentration of phosgene no reaction whatever was obtained until after several hours' standing.

The positive reaction for hydrochloric acid given by the benzidine test is doubtless due to the precipitation of benzidine hydrochloride. It was thought that the turbidity produced by phosgene might also be due to precipitation of the hydrochloride. To test this point a solution of benzidine in absolute chloroform was mixed with absolute chloroform containing about one per cent. of phosgene. The resulting precipitate was collected on a dry filter, well washed with absolute chloroform and then dried. It was found to be soluble in water and gave the ordinary reaction for chlorides. The yellowish white precipitate mentioned by Utz as being produced in the presence of much phosgene could not be obtained.

**SUMMARY.**—A new and simple method for detecting decomposition products in chloroform, due to phosgene formation, has been described. It has been shown that the barium hydroxide test is untrustworthy and that the benzidine test is not sufficiently sensitive for the requirements of a pharmacopoeia. The following new test is recommended:

To 15 c.c. of medicinal chloroform contained in a dry stoppered bottle of about 25 c.c. capacity add 20 mgrms. each of resorcinol and vanillin. Stopper, and when the reagents have dissolved, set aside in a dark place for one hour. Add 5 c.c. of 1 per cent. aqueous ammonia solution, shake and allow the mixture to separate. When there has been prior formation of phosgene or in the presence of hydrochloric acid a pink or red colour develops in the aqueous layer, reaching its full value in about 30 seconds; its intensity varies with the quantity of impurity present.

In conclusion I wish to thank Mr. T. T. Cocking, F.I.C., for his helpful criticism and advice, and the Directors of The British Drug Houses, Ltd., for permission to publish this work.

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## Contamination in Morphine Deposited in the British Pharmacopoeia Process for the Analysis of Opium.

By JITENDRA NATH RAKSHIT, F.I.C.

*(Read at the Meeting, May 6, 1931.)*

It was noted (ANALYST, 1919, **44**, 337; 1921, **46**, 482) that the British Pharmacopoeia method of determining morphine in opium is not very accurate. Hence it was thought advisable to find out what are the principal impurities in morphine deposited in this process, and how they can be eliminated.

A large sample of the mixed alkaloids was furnished by the crude morphine deposited in the B.P. (1914) process, being the residues from the analysis of nearly 10,000 samples of pure opium during the last five years. Two hundred grms. of this morphine were heated on a steam bath in a 4-litre flask with 200 c.c. of water, and about 150 c.c. of 30 per cent. (w/v) potassium hydroxide solution were added to dissolve the morphine. The alkaline solution was extracted 6 times with benzene, 500 c.c. being used each time. The last benzene extract did not leave any appreciable residue on evaporation. The united benzene extracts were filtered and distilled until about 100 c.c. were left in the distillation flask. This residual benzene, on slow evaporation, yielded crystals with the characteristics of codeine. The residue was dried for 6 hours in a steam oven, when it weighed 11.3 grms., i.e. 5.65 per cent. of the "B.P." morphine.

Five grms. of this dry benzene extract were dissolved in 200 c.c. of 1 per cent. (w/v) hydrochloric acid, heated on a steam bath for an hour and filtered. The filtrate was pink, indicating the presence of porphyroxine (Rakshit, *J. Chem. Soc.*, 1919, **59**, 455; *Ber.*, 1926, **115**, 2473). It was evaporated to dryness, powdered, and re-dissolved in 100 c.c. of the same dilute hydrochloric acid, and the solution was again evaporated to dryness. This process of solution in dilute hydrochloric acid and evaporation on a steam bath was repeated thrice more to complete the decomposition of the porphyroxine and some colouring matter. Finally, the dried salt was dissolved in 100 c.c. of hot water, and the solution was filtered and decolorised with 5 grms. of animal charcoal. The solution was heated again on a steam bath, filtered into a separator, made alkaline with 20 c.c. of 30 per cent. (w/v) potassium hydroxide solution, and thrice extracted with benzene, 100 c.c. being used each time. The united benzene extracts were evaporated until crystals formed. This crystalline residue was dried, powdered, and re-crystallised from 50 c.c. of 30 per cent. (w/v) alcohol, when 3.6 grms. (1st crop = 3.1 grms. and 2nd crop = 0.5 gm.) of well-formed codeine crystals were obtained.



They gave  $[\alpha]_D = -137.4^\circ$ ; m.pt.,  $155^\circ \text{C}$ . This sample, when mixed in equal quantities with codeine of known purity, did not alter in m.pt.

It is thus evident that morphine precipitated by the B.P. process is contaminated with about 3.6 per cent. of codeine. Of course, besides codeine, there are other alkaloids to make up the total of 5.65 per cent. in the impure morphine. In experiments to eliminate such foreign matter by exhaustion with a solvent, batches of 100 grms. each of mixed alkaloids, containing 92 per cent. of pure morphine and 8 per cent. of codeine, prepared from their hydrochlorides by precipitation with dilute ammonia from 1 per cent. aqueous solution, were heated on a steam bath with various solvents, and the solutions were filtered. All the filtrates were separately evaporated, and the residues were dried and weighed. The results of such repeated extractions with different solvents were as follows:

WEIGHTS IN GRMS. EXTRACTED BY DIFFERENT SOLVENTS FROM 100 GRMS. OF MIXTURES OF 92 PER CENT. OF MORPHINE AND 8 PER CENT. OF CODEINE.

No. of extractions.	Water = 500 c.c. Ammonia (10 per cent.) = 50 c.c. 550 c.c.		Water = 500 c.c. 90 per cent. alcohol = 50 c.c. 550 c.c.		Benzene = 500 c.c.
	Water = 500 c.c.				
1	3.40	2.90	1.85	3.04	
2	1.87	2.37	1.61	1.46	
3	1.51	2.45	1.50	0.52	
4	1.05	2.05	1.60	0.60	
5	0.86	1.50	1.11	0.57	
6	0.75	1.91	0.96	0.15	
7	0.51	1.96	0.95	0.07	
8	0.51	1.10	0.90	0.07	
9	0.65	1.78	0.81	0.06	
Total extracts	11.10	18.02	11.29	6.54	

Each of these total extracts was re-extracted separately with 50 c.c. of benzene, and the actual benzene extracts obtained were 1.61, 2.16, 2.40, and 5.06 grms. respectively.

The amounts of codeine in the main insoluble residues (chiefly morphine) left after repeated extractions were determined by extraction with benzene from potassium hydroxide solution, with the following results:—(1) 6.5 and 7.2 per cent.; (2) 6.7 and 7.2 per cent.; (3) 5.6 and 5.6 per cent.; and (4) 4.7 and 4.7 per cent.

It is thus evident that benzene removes some codeine from such mixtures, but that the results are far from satisfactory.

It seemed possible that extraction of a lime solution of opium, obtained, as in the B.P. process, with benzene prior to precipitation with ammonium chloride, might give a purer deposit of morphine, all other operations of the method remaining the same. Accordingly 100 c.c. of a lime solution of opium were prepared

according to the B.P. process, and shaken well with 100 c.c. of benzene in a separator for 30 minutes; the separated aqueous lower layer was filtered and 51 c.c. precipitated with ammonium chloride in the presence of alcohol and ether in accordance with the B.P. (1914) process. The same samples of opium were also analysed strictly according to the B.P. (1914) process. The following comparative results were obtained by the two methods:—

Sample Nos.	Morphine by	
	B.P. (1914) method. Per Cent.	B.P. (1914) method, as modified. Per Cent.
1	9.8	10.6
2	9.5	10.5
3	10.4	11.3

When morphine deposited by this modified process was dissolved in potassium hydroxide solution and extracted with benzene, as described above, it gave only 0.3 per cent. of substances soluble in benzene; therefore such morphine is much purer than that given by the original B.P. process.

Annett and Singh (ANALYST, 1918, 43, 208), when studying the effect of codeine in hindering the precipitation of morphine by ammonia from a solution of its lime compound, obtained deposits of morphine in the presence of codeine. It is not stated, however, whether such morphine was free from contamination by other bases. By preliminary extractions of a lime solution of opium with benzene a deposit of morphine cleaner and in greater quantity than usual is obtained. The higher yield need not interfere with the standard correction by 0.051 grm. (B.P. 1914) of the weight indicated by titration, since it has already been shown (Rakshit and D'Costa, ANALYST, 1919, 44, 337) that this correction figure is too low, at all events for Indian opium. If the B.P. process were modified in this way, the results would only approach the actual truth.

Dott (ANALYST, 1919, 44, 50) stated that sufficient ether is present to hold all codeine in solution, but in the presence of two immiscible layers the codeine seems to remain distributed between them in a certain ratio. Moreover, a good deal of ether [about 10 per cent. (v/v)] remains in the aqueous layer, which obviously increases the solubility of codeine in this layer. Such codeine, however, appears to become contaminated with morphine during its precipitation in the B.P. process.

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## The Use of Bromine as a Reagent in the Determination of Alkaloids.

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AND

R. G. O'BRIEN, *A. Austral. Chem. Inst.*

(Read at the Meeting, April 1st, 1931.)

**THALLEIOQUIN REACTION.**—The thalleioquin reaction for quinine has been dismissed as useless from a quantitative point of view (*cf. Allen's Commercial Organic Analysis*, 5th Ed., Vol. VII, p. 449; and W. B. Hart, *J. Soc. Chem. Ind.*, 1921, 72r), but in a critical examination of the test we have found that if it is carried out according to the procedure described below, it forms a simple and accurate method for the determination of this alkaloid. When it was found that the presence of many substances seriously interfered with the reaction it was elaborated and finally became an iodimetric determination.

In the course of the work new quantitative colour tests for other alkaloids were developed, bromine being used as a reagent in all cases. The most important of the tests recorded are those for codeine and emetine.

For convenience, the description of the investigation will be given in three groups:—(1) The cinchona alkaloids; (2) the opium alkaloids; (3) the ipecacuanha alkaloids.

With a view to elucidating the chemistry of the reactions obtained, some further work was undertaken with phenolic bodies. This is dealt with in Section IV of the paper.

### PART I. THE CINCHONA ALKALOIDS.

**THALLEIOQUIN REACTION.**—In the initial experiments with quinine it was noticed that an excess of halogen (bromine) impaired the delicacy of the thalleioquin reaction, the maximum colour being obtained when the halogen was just in excess. It was also found, in agreement with published procedure, that unless any large excess of bromine was removed the test was useless from a quantitative aspect. Whilst some success was obtained with other methods, it was quickly realised that aeration offered the best means of removing the excess.

**QUININE.**—The details of the method adopted for quinine are as follows:

The quinine salt (or quinine solution as nearly neutral as possible) is dissolved in 10 c.c. of water in a 25 c.c. graduated measuring cylinder. Four c.c. (or a suitable quantity) of  $N/5$  freshly prepared bromine water are added, and the

excess of bromine, after standing 1 minute, is expelled by passing 20 litres of air through the liquid by means of a glass tube with a fine orifice reaching to the bottom of the cylinder, the rate of aeration being approximately 1 litre per minute. The contents of the cylinder are then diluted to 20 c.c. with distilled water and 10 drops of 32.5 per cent. ammonia solution are added. The bulk is made up to 25 c.c. and agitated to ensure uniformity. The solution is set aside for 5 minutes to permit of the maximum colour developing, and the colour obtained is compared with a series of standards made with a solution of a pure quinine salt and treated in exactly the manner described. In actual estimations it is advisable not to use more than 0.02 gm. of quinine, owing to the frothing caused during aeration by the precipitated quinine and bromine compound. After aeration the liquid is clear and of a yellowish colour, the depth of colour being proportional to the quinine content of the solution. In testing, using a series of standards, the ammonia is not added until all the solutions have had bromine added and have been aerated. The colour is thus developed in the standards and test solution simultaneously.

It was found necessary, in comparing the colour obtained, to have standards containing approximately the same amount of quinine as the solution being investigated, as weak solutions of quinine develop a blue colour, whilst strong ones are green. When the colour of the solution has been approximately matched by Nesslerising, the standard whose colour most closely resembles the solution under examination is selected, and the accurate proportion of colour is determined by means of a Duboscq colorimeter. The quantity of quinine is then calculated. A series of standards containing 0.002, 0.005, 0.007, 0.010, 0.015, and 0.020 gm. of quinine was found to be convenient.

The aspirator used in the determination was a 5-litre jar, with the inlet tube reaching to the bottom, of sufficient length to permit, after disconnection from the water tap, of its being used as a syphon to draw off the water without disturbing the sealed cork of the jar. The outlet tube had a fine orifice and was long enough to reach to the bottom of the liquid being aerated. Four jars were used, and after each 5 litres of air had passed, the cylinder was washed down by sluicing some of the liquid into the bubbling tube and letting it run down.

The concentration of the excess bromine was varied over a fairly wide range, and the time of aeration also up to one hour (60 litres of air), but neither variation had any effect on the green colour produced. The time of standing after the addition of bromine and before aeration, however, is important.

In comparative tests with several salts of quinine, *viz.* the sulphate, acid sulphate, hydrochloride, and acid hydrochloride, the colours obtained were found to be strictly proportional to the quinine content of the various salts used.

Chlorine water was substituted for bromine, and although a deeper colour was obtained while using the same amount of quinine, its use is not recommended, as it was found more difficult to free the solution from excess of halogen, and, also, because the chlorine reagent is usually more acid than bromine. This is a disadvantage, as will be seen later.

The delicacy of the test, if carried out as described, is approximately 1 part of alkaloid in 50,000 parts of water, using 50 c.c. of solution. This is less sensitive than the maximum delicacy of the test where a bare excess of bromine is present, when a concentration of 1 part of alkaloid in 150,000 is just detectable. Experiments showed that the maximum delicacy is reached when four atoms of bromine are attached to one molecule of alkaloid, one of them being removable by aeration. For testing purposes, however, aeration is recommended, as uniform conditions are easily obtainable and the test is, therefore, under control. A slight excess of bromine above the 4 atoms mentioned is inimical to the test, and, in working with concentrations such as 0.0001 grm. of alkaloid in 10 c.c. of solution, ideal bromine conditions are hard to obtain other than by the aeration process described.

As, except in the testing of the various pharmacopoeial salts, a pure quinine solution is rarely met in practice, experiments were continued to investigate the influence of substances usually associated with this alkaloid. It was found that neutral salts such as sodium sulphate, in moderate concentration, do not interfere, but that ethyl alcohol has a marked influence, as is shown by Table I.

(a) QUININE IN PRESENCE OF ETHYL ALCOHOL.—To vary the alcoholic strength, different proportions of (1) 0.1 per cent. solution of quinine (as sulphate) in water, and (2) 0.1 per cent. solution of quinine (as sulphate) in absolute alcohol were used, aqueous standards of quinine salt being employed for the comparison.

TABLE I.

Quinine taken. Grm.	Alcoholic strength of solution. Per Cent. (by vol.).	Quinine found. Grm.	Percentage found.
0.005	Nil	0.005	100
0.005	10	0.0044	88
0.005	20	0.0033	66
0.005	45	0.0008	16
0.005	75	Nil	—

The results show that the presence of even 10 per cent. (by vol.) of ethyl alcohol renders the method inaccurate, unless the standards are prepared with a quinine solution of an alcoholic strength exactly similar to that of the test. The alcohol can, of course, be removed by distillation, the residue made up to a sufficient bulk, and a suitable quantity tested as before described. The explanation of the failure of the test lies in the fact that dilute alcohol retains some of the excess bromine that cannot be expelled by aeration, and at a concentration of 70 per cent. of alcohol (by vol.), this excess is sufficient to mask even a qualitative reaction with 0.005 grm. of the alkaloid in 15 c.c. of solution.

(b) THE EFFECT OF FREE ACID.—Free acid (HCl) has a very marked effect on the strength of colour obtained. With a solution of 0.005 grm. of quinine in 10 c.c. of *N*/10 acid, not more than 20 per cent. of the colour was obtained. In 10 c.c. of *N*/5 acid, less than 10 per cent. of the colour was obtained. If, however, the

free acid is almost neutralised with sodium hydroxide before adding the bromine, the reaction is satisfactory.

(c) IN PRESENCE OF CINCHONIDINE.—When 0.005 grm. quantities of each alkaloid in 15 c.c. of solution were taken, practically 100 per cent. yield was obtained, excepting that the colour was rather greener than when the quinine was used alone, and hence was difficult to match. In the presence of much cinchonine or cinchonidine, standards should be prepared containing approximately the same amounts of these substances.

(d) IN PRESENCE OF STRYCHNINE.—Two solutions were used throughout this series of experiments in order to obtain the various mixtures used:—(1) 0.1 per cent. solution of quinine sulphate; (2) 0.1 per cent. solution of strychnine.

TABLE II.

Quinine taken. Grm.	Strychnine taken. Grm.	Quinine found. Grm.	Percentage of quinine found.
0.005	Nil	0.005	100
0.005	0.0025	0.005	100
0.005	0.005	0.0045	90
0.005	0.010	0.0042	84
0.005	0.015	0.0036	72

After aeration, 10 c.c. of 90 per cent. alcohol were added before making up to 25 c.c. with distilled water. This is necessary, as the addition of ammonium hydroxide precipitates strychnine, which must be dissolved before the colour can be compared. The standards were treated in the same way. It will be seen that, although small quantities of strychnine do not interfere with the colour, as the proportion of strychnine increases the method becomes useless for purposes of determination.

In order to overcome the interference of large quantities of strychnine, the following method was used, 0.05 grm. of strychnine and 0.002 grm. of quinine being taken. These amounts were dissolved in chloroform and the solution was evaporated to dryness in a 7 cm. glass crystallising dish in order to get a mixture of alkaloids such as would be obtained after estimation in analytical practice. The dry alkaloids were treated with 10 c.c. of ethyl ether, and rubbed with a glass rod, and the liquid was filtered through a 5 cm. paper. The residue remaining in the dish, and the rod, were treated with 5 c.c. of ethyl ether and filtered through the same paper. The total filtrate was evaporated in a 5 cm. glass crystallising dish. The residue after evaporation (quinine) was dissolved in 1 c.c. of  $N/10$  hydrochloric acid, evaporated to dryness, and dissolved in 8 c.c. of hot water. The dish was washed with 2 c.c. of hot water and the contents were transferred to a 25 c.c. glass cylinder and cooled to  $20^{\circ}\text{C}$ . An excess of  $N/5$  bromine was added, and the solution was aerated as described, treated with 10 drops of 32.5 per cent. ammonia solution, and made up to 25 c.c. with distilled water; 0.002 grm. of quinine in 15 c.c. solution was placed in a 25 c.c. cylinder, treated

with  $N/5$  bromine, aerated, and the colours compared. The quinine found in the ethereal extract of the mixed alkaloids amounted to 0.0019 grm. It is thus seen that the colorimetric method for quinine is applicable, even in the presence of comparatively large quantities of strychnine, if the latter alkaloid is removed (*cf.* A. R. Bliss, *J. Amer. Pharm. Assoc.*, 1919, 804).

The method for the separation of quinine and strychnine described by Norman Evers (*Pharm. J.*, 1922, p. 90), was also investigated at this stage and found to be an excellent one. The quinine obtained by the use of this method was estimated colorimetrically and found to be pure. No reaction for quinine could be obtained with the strychnine separated.

(e) QUININE AND CAFFEINE.—Experiments with quinine in the presence of caffeine showed that when 0.002 grm. of quinine was mixed with 0.02 grm. of caffeine no thalleioquin reaction was obtained after aeration with bromine and addition of ammonium hydroxide.

As, from the foregoing experiments, the colour reaction for the determination of quinine was found to have a limited use, and as definite evidence had been obtained of a stable bromo-quinine compound, it was thought that by the addition of potassium iodide to the solution, after treatment with bromine and aeration, it would be possible to titrate the liberated iodine or iodo compound with thio-sulphate solution and so make the determination volumetric. Experiments of this nature, as seen from Table III, gave satisfactory results, the tests being carried out at a temperature of 25° C. This is an important point.

The method used was identical with the one previously described for the colorimetric determination of quinine, excepting that a small amount of potassium iodide (approximately 0.5 grm.) was substituted for the 32.5 per cent. ammonia solution, and the quinine-iodo compound was titrated, without filtration, with  $N/100$  thiosulphate solution, a 2 per cent. solution of clear soluble starch being used as indicator.

TABLE III.

Quinine used (alkaloid). Grm.	$N/100$ thiosulphate solution required. c.c.
0.003	1.75
0.006	3.60
0.010	5.95
0.015	8.9

From these results it was concluded that 1 c.c. of  $N/100$  thiosulphate solution corresponds with 0.00166 grm. of anhydrous quinine (0.00162 grm., equivalent to 2 atoms of iodine, being liberated from 1 molecule alkaloid by reaction to potassium iodide and forming a di-iodo-quinine compound).

Various amounts of strychnine (from 0.001 to 0.010 grm.) were treated in the same way, but no iodine was liberated. When  $N/5$  bromine was added to the strychnine solution a bromine compound of strychnine was precipitated in each

case, but where the strychnine was less than 0.006 grm. in 15 c.c. of solution it dissolved on aeration. Even when it remained undissolved no liberation of iodine was observed after aeration on testing with potassium iodide and starch solution.

TABLE IV.

EFFECT OF TEMPERATURE ON IODIMETRIC DETERMINATION OF QUININE.

Quinine taken. Grm.	Temperature of test solution °C.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.	Remarks.
0.006	0°	2.5	0.0041	Experiment carried out as described
0.006	0°	2.95	0.0049	Solution allowed to stand with excess of bromine for 30 mins. before aeration
0.006	15–16°	3.35	0.0056	Solution allowed to stand 15 mins. after addition of bromine before aeration
0.006	23–25°	3.55	0.0059	Solution allowed to stand 1 min. after addition of bromine before aeration
0.006	28°	3.55	0.0059	Do. do. do.
0.006	40°	3.4	0.0056	Do. do. do.
0.006	50°	3.4	0.0056	Do. do. do.

A temperature of 25 to 28° C. is most favourable for the maximum liberation of iodine, and this temperature was, therefore, used throughout the work detailed in this paper. At this temperature, on the addition of potassium iodide, 2 atoms of iodine are liberated and can be titrated with thiosulphate solution.

TABLE V.

QUININE AND STRYCHNINE: IODINE METHOD.

Quinine taken. Grm.	Strychnine taken. Grm.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.	Remarks.
0.005	Nil	3.05	0.00506	Cleared after aeration
0.010	0.001	6.05	0.01004	Do. do.
0.005	0.002	3.05	0.00506	Do. do.
0.005	0.005	3.1	0.00514	Did not clear completely on aeration. Titrated slowly, as precipitate seemed to dissolve slowly
0.005	0.010	3.1	0.00514	Do. do. do.

It is seen that the method yielded good results with a mixture of quinine and strychnine.

END-POINT OF TITRATION WITH MIXTURES OF QUININE AND STRYCHNINE.—The titration should be carried out slowly. The starch should be added approximately at the stage of 1 c.c. before the end of the titration with N/100 thiosulphate



solution. The solution at this stage has a slight precipitate of the quinine-strychnine-iodine compound and is very yellow. On the addition of starch, the solution becomes blue-green. The *N*/100 thiosulphate solution is added, 2 drops at a time, and the solution gradually loses its colour, becoming yellow, and also clears appreciably. The end-point is taken when no white rings are visible on the addition of 2 drops of *N*/100 thiosulphate solution. The end-point is very noticeable on looking at the solution against a white ground immediately after the addition of the thiosulphate. The quinine compound appears to give up its iodine very slowly in the presence of strychnine. On this account the titration rate near the end-point is about 2 drops in 15 seconds. The starch must be clear and freshly made, and must not be added too early in the titration.

ALTERNATIVE END-POINT METHOD.—If, after the aeration and addition of potassium iodide, a slight excess of *N*/100 thiosulphate solution is added and the test is put aside for five minutes in order to permit the mixed iodine compounds to dissolve, and the slight excess of thiosulphate is then titrated with *N*/100 iodine solution; a sharp end-point is obtained. In carrying out this test for a mixture care must be taken to observe the following conditions:—(1) After the addition of bromine not more than about a minute should be allowed to elapse before aeration is begun, as otherwise the precipitated strychnine and bromine compound settles and is difficult to get into solution; and (2) a fair excess of bromine must be added before aeration. If this is not done, the strychnine may deprive the quinine of some of its bromine and the result will be low.

TABLE VI.

QUININE AND CAFFEINE TESTED IN 15 C.C. OF SOLUTION AT A TEMPERATURE OF 25° C.

Quinine taken. Grm.	Caffeine taken. Grm.	<i>N</i> /100 thiosulphate solution required. c.c.	Quinine found. Grm.	Remarks.		
0.003	Nil	1.75	0.0029	Experiment carried out as described		
0.003	0.005	1.7	0.0028	Do.	do.	do.
0.003	0.015	1.3	0.0021	Do.	do.	do.
0.003	0.025	0.75	0.0012	Do.	do.	do.
0.003	0.050	0.45	0.0007	Do.	do.	do.
0.003	0.100	0.15	0.0002	Do.	do.	do.
0.003	0.025	1.75	0.0028	Allowed to stand 10 minutes after addition of bromine before aeration		
0.006	0.025	3.55	0.0059	Do.	do.	do.

In the presence of much caffeine it is necessary to add a large excess of bromine and to allow the solution to stand for 10 minutes before aeration, as otherwise the caffeine absorbs the bromine at the expense of the quinine, and it is necessary, therefore, to saturate both. If these conditions are observed, the method is quite

good for comparatively small amounts of quinine in the presence of large amounts of caffeine. If desired, however, the caffeine can be separated from the quinine by extraction with chloroform from acid solution before making the test on the quinine.

Caffeine in the form of citrate was found to behave like the free alkaloid.

TABLE VII.

AMOUNT OF BROMINE REQUIRED FOR DETERMINATION OF QUININE IN MIXTURES OF QUININE AND CAFFEINE.

Quinine taken. Grm.	Caffeine taken. Grm.	N/5 bromine added. c.c.	N/100 thiosulphate solution required. c.c.	Remarks.		
0.006	nil	0.6	2.75	Not sufficient bromine added		
0.006	nil	1.0	3.6	No standing after addition of bromine before aeration		
0.006	nil	1.0	3.6	Allowed to stand 10 minutes before aeration		
0.006	nil	2.0	3.6	No standing before aeration		
0.006	nil	2.0	3.55	Allowed to stand 10 minutes before aeration		
0.006	0.025	2.0	2.6	Colour disappeared on standing 3 minutes; insufficient bromine		
0.006	0.025	4.0	3.0	Allowed to stand 10 minutes before aeration		
0.006	0.025	6.0	3.5	Do.	do.	do.
0.006	0.025	8.0	3.55	Do.	do.	do.

Table VII shows that in the presence of caffeine care must be taken to have an excess of bromine present. One c.c. of N/5 bromine should be present for every 0.006 gm. of quinine, and for every 0.003 gm. of caffeine. Although none of the absorbed bromine in the case of caffeine reacts on the addition of potassium iodide, it seems necessary to saturate this alkaloid, or it will deprive the quinine of its more loosely combined halogen, and a low result will be obtained.

TABLE VIII.

QUININE IN PRESENCE OF COCAINE.

Quinine taken. Grm.	Cocaine taken. Grm.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.
0.006	nil	3.55	0.0059
0.006	0.005	3.55	0.0059
0.006	0.02	3.6	0.0060

TABLE IX.

## QUININE IN PRESENCE OF ATROPINE.

Quinine taken. Grm.	Atropine taken. Grm.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.
0.006	nil	3.6	0.0060
0.006	0.02	3.6	0.0060
0.006	0.05	6.3	0.0060

TABLE X.

## QUININE IN PRESENCE OF CINCHONINE.

Quinine taken. Grm.	Cinchonine taken. Grm.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.
0.006	nil	3.55	0.0059
0.006	0.005	3.6	0.0060
0.006	0.010	3.6	0.0060

TABLE XI.

## QUININE IN PRESENCE OF CINCHONIDINE.

Quinine taken. Grm.	Cinchonidine taken. Grm.	N/100 thiosulphate solution required. c.c.	Quinine found. Grm.
nil	0.010	nil	—
0.006	nil	3.5	0.0059
0.006	0.010	3.7	0.0062

From Tables VIII, IX, X, and XI it will be seen that the method can be used to determine quinine in the presence of cocaine, atropine, cinchonine and cinchonidine.

OTHER CINCHONA ALKALOIDS.—As the reaction permitted of the satisfactory determination of quinine in a mixture of other alkaloids, it was thought that the other cinchona alkaloids giving the thalleioquin reaction would also be capable of determination in a similar manner to quinine. The reaction was tested on cupreine, with satisfactory results, as shown in Table XII.

TABLE XII.

Cupreine Grm.	N/100 thiosulphate solution required. c.c.
0.006	3.75
0.010	6.3

Thus, 1 c.c. of *N*/100 thiosulphate solution equals 0.00158 grm. of anhydrous cupreine. Theoretically 1 c.c. should equal 0.00155 grm.

It seems most probable that the reaction would also be given by quinidine, hydroquinine, hydroquinidine and diquinicin, but these have not yet been tested.

**SUMMARY OF WORK ON CINCHONA ALKALOIDS.**—(1) The thalleioquin reaction has been made quantitative for the determination of quinine and its salts.

(2) The quantitative reaction is unreliable in the presence of such substances as free acid, alcohol, and various alkaloids, *e.g.* strychnine, and caffeine.

(3) An iodimetric development of the method is described which permits of the estimation of quinine in the presence of other alkaloids, *viz.* strychnine, caffeine, cocaine, atropine, etc.

(4) The method has been applied to cupreine, with satisfactory results, and it is thought that it would be applicable to those other members of the cinchona group which give the thalleioquin reaction, such as quinidine, hydroquinine, etc.

## PART II. THE OPIUM ALKALOIDS.

During the preceding investigation it was observed that the presence of morphine seriously interfered with the thalleioquin reaction, and, on applying the iodine development of this test, it was found that, if suitable conditions were observed, morphine reacted in a somewhat similar manner to quinine, *i.e.* 2 atoms of iodine were liberated on addition of potassium iodide to the aerated bromine compound. The tests described in Table XIII were conducted at a temperature of 25° to 28° C., with a view to determining the details of the method to be adopted for the determination, the morphine being present as hydrochloride.

TABLE XIII.

Morphine (alkaloid). Grm.	Time of standing after aeration and addition of potassium iodide.	Quantity of acid added after aeration	<i>N</i> /100 thiosulphate solution required. c.c.	Remarks.
0.005	nil	nil	2.4	Indefinite end-point; comes back
0.005	5 mins.	nil	2.8	Do. do.
0.005	5 ..	1 c.c. of <i>N</i> /10 HCl	3.1	Do. do.
0.005	15 ..	1 c.c. do.	3.26	Do. do.
0.005	15 ..	1 c.c. of <i>N</i> /1 HCl	3.4	Fairly good end-point
0.005	30 ..	1 c.c. do.	3.45	Do. do.
0.005	60 ..	2 c.c. do.	3.55	Satisfactory end-point
0.010	60 ..	2 c.c. do.	7.15	Do. do.

From the results obtained satisfactory details of the application of the test were evolved as follows:

The alkaloid in the form of a salt in 15 c.c. of a solution, is made as nearly neutral as possible, placed in a 25 c.c. graduated measuring cylinder, and treated with an excess of  $N/5$  bromine (freshly prepared). After 1 minute the excess of halogen is removed by aeration, as described for quinine (20 litres of air passed through at the rate of approximately 1 litre per minute). After aeration, 2 c.c. of  $N$  hydrochloric acid and excess of potassium iodide (approximately 0.5 gm.) are added, and the corked solution is set aside for 1 hour. A few drops of clear freshly prepared starch solution are then added, and the liberated iodine is titrated with  $N/100$  thiosulphate solution. A good end-point is obtained.

Strict attention must be paid to the time of standing, the temperature, and the concentration of the acid used. The liberation of iodine corresponds with 2 atoms of iodine, being liberated by the morphine-bromine compound, after aeration, on the addition of potassium iodide. Therefore, 1 c.c. of thiosulphate solution equals 0.00142 gm. of alkaloid.

In an actual test, a 2.5 per cent. solution of morphine hydrochloride, as submitted to the laboratory for the determination of the alkaloidal strength, was taken. One c.c. of this solution was diluted to 15 c.c. with distilled water and tested as outlined above. It required 12.9 c.c. of  $N/100$  thiosulphate solution, equivalent to 0.0244 gm. of morphine hydrochloride (containing 3 molecules of water of crystallisation). Morphine and atropine tablets were also tested by this method with excellent results, the atropine present apparently making no difference in the determination of the morphine.

**DIFFERENCES OBSERVED IN QUININE AND MORPHINE REACTIONS.**—Morphine in solution at the concentration tried, does not give a bromine alkaloid precipitate. After aeration the solution is of a very much more pronounced yellow colour than a quinine solution of the same strength.

**DETERMINATION OF QUININE AND MORPHINE MIXTURE.**—By comparison of the yellow colour obtained after aeration of the bromine alkaloid compound with standards prepared in the same manner, it was found that the morphine could be determined with an accuracy of 5 per cent. of the amount present. In a mixture of quinine and morphine the titration of the liberated iodine gives the total alkaloid. By varying the proportions of quinine and morphine within this amount and comparing the yellow colour produced after the addition of bromine and aeration, the quantity of each alkaloid could be determined.

A further development of this test is as follows:—On the addition of slight excess of  $N/5$  bromine solution and then of excess of hydrogen peroxide (3 per cent. solution) to a dilute morphine solution, the excess of bromine is removed, but the solution remains yellow in proportion to the quantity of morphine present. This is determined by taking 0.5, 1, 1.5, 2 and 2.5 c.c. of a solution containing 0.001 gm. of morphine per c.c. in matched test tubes, and, after making up to 3 c.c. with distilled water, adding 2 to 3 drops in excess of  $N/5$  bromine, than 1 c.c. of 3 per cent. hydrogen peroxide, setting aside for 2 minutes with occasional shaking, and then transferring with washing to a Nessler glass, making up to 50 c.c. with

distilled water, and comparing the colours produced. On adding, drop by drop, *N*/10 sodium hydroxide solution, or very dilute ammonium hydroxide (5 drops of 0.4 *N*), a red colour develops which is proportional to the amount of morphine present. Dilute alkali must be used, as the colour fades in the presence of strong alkali.

*Codeine.*—Codeine does not give a yellow colour with bromine and hydrogen peroxide, but develops a red colour after the addition of alkali. Table XIV shows the behaviour of common alkaloids in the test, 0.003 grm. of alkaloid to 3 c.c. of solution being taken and treated with bromine and peroxide as described above.

TABLE XIV.

Alkaloid 0.003 grm.	Yellow colour with <i>N</i> /5 bromine and hydrogen peroxide.	Red colour with alkali.
Morphine	Yes	Yes
Codeine	No	Yes
Strychnine	No	Very faint; rapidly disappears
Quinine	Faint; one-tenth that of morphine	No; becomes yellow
Veratrine	No	No
Atropine	No	No
Cinchonidine	No	No
Narcotine	Slight on filtration	No, but alkaloid precipitates
Narceine	No	Strong yellow, suggesting orange
Thebaine	No	Slight pink
Papaverine	Slight yellow. Heavy precipitate; does not clear with hydrogen peroxide until warmed.	No
Meconic acid	No colour	No
Emetine	Yes	No
Cephaeline	Yes	No

The presence of various alkaloids in admixture with morphine, did not seem to interfere with the depth of colour produced, *i.e.* 0.002 grm. of morphine, plus 0.002 grm. of strychnine, gave the same colour as 0.002 grm. of morphine alone. By means of the combined tests described it was often possible to determine the percentage of three alkaloids in a mixture.

Returning to the bromine aeration test, Table XV shows the behaviour of morphine in the presence of quinine, the test being carried out as described for morphine.

TABLE XV.

Morphine taken. Grm.	Quinine taken. Grm.	<i>N</i> /100 thiosulphate solution required. c.c.
nil	0.006	3.6
0.005	nil	3.55
0.005	0.006	7.15

Table XVI gives the results obtained with a mixture of morphine and strychnine.

TABLE XVI.

Morphine taken. Grm.	Strychnine taken. Grm.	N/100 thiosulphate solution required. c.c.
0.003	0.005	2.3
0.005	0.005	3.7

In the presence of strychnine, the morphine result will be a little on the high side (0.005 grm. being estimated as 0.0052 grm.). The same conditions must be observed with a morphine and strychnine mixture as were described for the quinine and strychnine mixture in regard to the end-point of the titration.

CODEINE.—To the dilute solution of codeine salt, excess of *N*/5 bromine is added, and the solution is aerated as described for morphine. Two c.c. of *N* hydrochloric acid and potassium iodide solution are then added, and the solution is set aside for 60 minutes. A slight excess of *N*/100 thiosulphate solution is added, and the solutions are allowed to stand for 3 minutes. The excess of thiosulphate is titrated with *N*/100 iodine (starch as indicator), the experiment being carried out at 28° C.

TABLE XVII.

Codeine taken. Grm.	N/100 thiosulphate solution required. c.c.
0.005	3.3
0.01	6.5
0.015	9.9

Codeine behaves similarly to morphine, *i.e.* 2 atoms of iodine are liberated after the addition of bromine, and aeration, and the addition of potassium iodide and *N* hydrochloric acid.

DIFFERENCES OBSERVED BETWEEN CODEINE AND MORPHINE.—At the dilution used codeine gives a precipitate with excess of bromine; morphine does not. On the addition of potassium iodide after aeration, codeine gives a precipitate; morphine does not. After aeration the colour of a codeine solution is pink in proportion to the quantity of alkaloid present. This colour disappears on the addition of acid, leaving the solution a very slight yellow. The pink coloration was confirmed with several codeine salts, *e.g.* the phosphate and hydrochloride. After passing the first 5 litres of air, no colour was noticed; with 10 litres passed, a slight pink coloration was obtained; with 15 litres, the intensity was much increased. The effect of oxidation observed on the codeine and bromine compound in this experiment led us to try the effect of other oxidising agents, and a new colorimetric determination for codeine was developed as follows:

The solution of codeine phosphate contained in 1 c.c. the equivalent of 0.001 grm. of codeine. Quantities of 0.5, 1.0, 1.5, 2 and 2.5 c.c. of this solution

were placed in matched 25 c.c. test tubes, and each made up to 3 c.c. with distilled water. A slight excess of  $N/5$  bromine was added, drop by drop, until the yellow colour persisted, about 2 drops excess being added. One c.c. of 3 per cent. hydrogen peroxide was added to each tube, and they were then placed in a water bath at a temperature of  $70^{\circ}$  to  $80^{\circ}$  C. for 6 minutes, when the maximum pink colour developed. The solutions were cooled, transferred with wash water to a 50 c.c. Nessler glass, and made up to 50 c.c. with distilled water, and the colours were compared. The depths of colour were found to be strictly proportional to the amounts of codeine present. The limit of the test was found to be 1 part in 25,000 of alkaloid, when using 2 c.c. of this solution for the test, 2 drops of  $N/5$  bromine and 1 c.c. of 3 per cent. hydrogen peroxide. The solution should be practically neutral before testing.

**EFFECTS OF OTHER ALKALOIDS ON THE TEST.**—Morphine and codeine, when mixed, give an orange colour due to the red of the codeine and the yellow of the morphine. Knowing the total concentration of the alkaloid in the solution as determined iodimetrically, it was found possible to estimate the percentages of morphine and codeine from this colour.

Strychnine in the proportion 0.0025 grm. to 0.005 grm. of codeine did not interfere.

Quinine interferes, as is shown by the following results:

Codeine	..	0.0005 grm.:	Quinine	..	0.0025	No pink coloration.
"	..	0.001	"	..	0.001	Positive, but reduced reaction.
"	..	0.001	"	..	0.002	Faint coloration just noticeable.

The colorimetric determination of morphine and codeine in a mixture can be made, as described previously, by using the yellow colour developed on the addition of bromine and peroxide for the determination of the morphine, and the red colour on the addition of alkali for the determination of the total alkaloid.

**NARCEINE.**—The method of determination was similar to that for morphine; 0.015 grm. of the alkaloid in the form of hydrochloride required 10.9 c.c. of  $N/100$  thiosulphate solution, and 0.010 grm. required 7.2 c.c. of  $N/100$  thiosulphate. From these results it was concluded that the equivalent of 3 atoms of iodine is liberated on the addition of potassium iodide to the narceine and bromine compound after aeration.

**THEBAINE.**—On treating a solution of 0.005 grm. of the alkaloid in the form of hydrochloride in the same way as for morphine, 5.0 c.c. of  $N/100$  thiosulphate were required; 0.010 grm. required 9.9 c.c. of  $N/100$  thiosulphate. From these results it was concluded that the equivalent of 3 atoms of iodine is liberated on the addition of potassium iodide to the thebaine and bromine compound after aeration.

**PAPAVERINE.**—On the addition of bromine to a dilute aqueous solution of a salt of the alkaloid, a copious precipitate forms which does not clear on aeration. The method described is not satisfactory for the determination of this alkaloid.



**NARCOTINE.**—On the addition of bromine to a dilute aqueous solution of a salt of the alkaloid, a copious precipitate forms which does not clear on aeration. The method described is not satisfactory for the determination of this alkaloid. From the results of the experiment with papaverine and narcotine, it would appear that neither of these alkaloids reacts like morphine. Only a very small liberation of iodine from the bromine and alkaloid compound results on the addition of potassium iodide to either of these substances.‡

**MECONIC ACID.**—Although good end-points were obtained, on treating dilute solutions of meconic acid in the manner described for morphine, some irregularity was observed in the amount of *N*/100 thiosulphate solution required. It was concluded that in order to obtain concordant results with this substance, a period of more than one minute must elapse after the addition of *N*/5 bromine before the aeration. Very little increase in the quantity of thiosulphate solution used was observed after 5 minutes' standing, as Table XVIII shows, and, therefore, this time was adopted as the most suitable for the estimation.

TABLE XVIII.

Meconic acid taken. Grm.	Time of standing with excess of <i>N</i> /5 bromine before aeration. Minutes.	<i>N</i> /100 thiosulphate required. c.c.
0.005	1	3.5
0.005	5	4.1
0.005	10	4.2
0.005	30	4.3
0.010	5	8.3

Although within this time period there is no definite molecular liberation of iodine, consistent results are obtainable, and 1 c.c. of *N*/100 thiosulphate is equivalent to 0.00120 gm. of meconic acid. With this modification, the determination of meconic acid can be carried out as described for morphine.

**MORPHINE DERIVATIVES AND SUBSTITUTES.**—*Heroin.*—To an aqueous solution of heroin hydrochloride containing 0.015 gm. of anhydrous heroin, excess of *N*/5 bromine was added, and the test carried out as described for morphine, 2.8 c.c. of *N*/100 thiosulphate being required; 0.005 gm. of heroin required 0.9 c.c.

It was, therefore, decided that, although heroin does not give an exact molecular liberation of iodine under the conditions of the test, comparative results are obtainable. It was further observed that the bromine compound, after aeration, has not the yellow colour of the morphine compound. It was also found that, after the addition of slight excess of bromine, hydrogen peroxide and dilute alkali, as described in the determination of morphine, a red coloration was obtained.

*Apomorphine.*—The solution contained in 1 c.c. apomorphine hydrochloride equivalent to 0.001 gm. apomorphine. On the addition of *N*/5 bromine to a dilute aqueous solution, a strong red colour, or red precipitate, is given according

to the concentration of the solution. This precipitate does not dissolve on aeration, and, therefore, the iodo-volumetric method of determination is useless. The following procedure, however, gave a satisfactory colorimetric determination:

To a series of very dilute solutions containing, respectively, 0.0002, 0.0004, 0.0007, and 0.001 grm. of alkaloid in 3 c.c. of solution,  $N/5$  bromine was added, drop by drop, until 2 or 3 drops in excess. This excess can be readily observed by the yellowing of the red colour produced. One c.c. of 3 per cent. hydrogen peroxide was then added to destroy the excess of bromine. It was found that the solutions were coloured red in proportion to the quantity of alkaloid present. This series of standards diluted to 50 c.c. with distilled water, was used to determine the quantity of alkaloid in an unknown solution of apomorphine treated in exactly the manner described. When the test was carried out in matched test tubes, using the same amounts of alkaloid as previously, and, after the addition of  $N/5$  bromine and hydrogen peroxide, the tubes were heated to approximately  $95^{\circ}\text{C}$ . by immersion in a water-bath for exactly four minutes, it was found that the red colour was changed to a permanent green, the depth of which was proportional to the amount of alkaloid present. The solutions were then cooled and diluted to 50 c.c., and Nessler glasses were used for the comparison of colour. As the time of heating is important, both the standards and the sample under examination should be heated for exactly the same time in the same bath.

*Ethyl morphine.*—The volumetric method described for morphine is suitable for the determination of this derivative, 0.005 grm. requiring 3.1 c.c. of  $N/100$  thiosulphate. The bromine derivative, after aeration, is not yellow, in this respect differing from morphine. On the addition of  $N/5$  bromine, hydrogen peroxide and dilute alkali, a colour similar to that given by morphine develops. This substance was the only one encountered during this investigation which gave the same pink reaction as codeine on treatment with bromine and hydrogen peroxide and heating to  $70^{\circ}\text{C}$ . for six minutes. This test, if carried out as described for codeine, can be used for the quantitative colorimetric estimation of ethyl morphine.

**EXPERIMENTS WITH OPIUM.**—With the idea of using the volumetric method described as a means of determining the amount of morphine and codeine in opium, an artificial solution of opium was prepared, containing, in the form of salts, 0.10 grm. of morphine, 0.06 grm. of narcotine, 0.05 grm. of meconic acid, 0.01 grm. of papaverine, 0.005 grm. of codeine, and 0.005 grm. of thebaine. This solution was made up to 25 c.c. with distilled water, and made slightly acid to litmus with  $N/10$  hydrochloric acid. The solution was then extracted with 10 c.c. of chloroform, and a further 5 c.c. of chloroform, each portion of chloroform being washed in succession with 5 c.c. of distilled water. The wash water was added to the original solution. The chloroform was evaporated to dryness, and the residue was weighed (0.0724 grm.). This represented narcotine, papaverine and thebaine. No reaction with formaldehyde and sulphuric acid could be obtained on the extract, showing the absence of morphine. A very slight reaction for meconic acid was obtained with ferric chloride. To the warm aqueous solution, after extraction

with chloroform, was added, drop by drop, 1.5 c.c. of 10 per cent. neutral lead acetate solution, this precipitating the meconic acid as lead meconate. The bulk of the liquid and precipitate was now made up to 45 c.c. with distilled water, and 40 c.c. of this were filtered, representing eight-ninths of the original solution. Hydrogen sulphide was passed through the warm filtrate for ten minutes. The precipitated lead sulphide was filtered off on a 7 cm. paper, and the filtrate was collected in a 100 c.c. graduated cylinder. The precipitate was washed three times with 10 c.c. of distilled water, and the total bulk of the solution, when cool, was made up to 89 c.c. with distilled water. Ten c.c. of this solution (equal to one-tenth of the original morphine and codeine added) after aeration with 10 litres of air to expel any hydrogen sulphide, were treated with excess of *N*/5 bromine and aerated as described for morphine. Potassium iodide and 2 c.c. of *N* hydrochloric acid were added, and the solution was set aside for one hour; 7.8 c.c. of *N*/100 thiosulphate solution were required for titration. This result is fairly good, as the quantities of morphine and codeine present should have required 7.5 c.c. Experiments with powdered opium, however, were not so satisfactory, the chief difficulty experienced being the solution of the alkaloids. Investigation is now proceeding on this point, and it is hoped shortly to describe a quick, reliable volumetric method for the determination of morphine and codeine in opium.

**SUMMARY OF THE WORK ON OPIUM ALKALOIDS.**—(1) A volumetric method is described, involving the use of the bromine and morphine compound obtained after aeration with excess bromine. From this compound the iodine liberated on the addition of potassium iodide is determined by means of thiosulphate solution. Codeine, narceine, thebaine and meconic acid can also be determined by this method. Narcotine and papaverine do not give satisfactory results, probably owing to the insolubility of the bromine compound formed. The method is applicable to the estimation of morphine in tablets, etc.

(2) A new colour reaction is described for the detection and determination of codeine, depending on the depth of the red colour formed by the oxidation of the bromine compound with hydrogen peroxide, and warming to 60° to 70° C. Ethyl-morphine also gives this reaction.

(3) A method is described for the determination of morphine and codeine, depending on the yellow colour developed by morphine after the addition of bromine and hydrogen peroxide. On the addition of dilute alkali a red coloration, proportional to the amount of alkaloid present, is obtained.

### PART III. THE IPECACUANHA ALKALOIDS.

**EMETINE.**—This alkaloid, tested in a manner similar to that described for the determination of morphine, behaved, under definite conditions, as if 2 atoms of iodine were liberated on the addition of potassium iodide to the aerated bromine and alkaloid compound, but the method, on further investigation, did not prove accurately quantitative. Much frothing was observed during the aeration, especially after the greater part of the excess of bromine had been removed. This

was apparently produced by the formation of a saponin-like substance during the course of the reaction. As the bromine is slowly removed by aeration the solution becomes colourless. After the decolorisation is practically complete, a deep yellow colour, very much more intense than that of the morphine and bromine compound, develops. This colour reaches its maximum after 20 to 25 litres of air have been passed. The intensity of the yellow colour was found to be proportional to the amount of alkaloid present, and could be used for its determination if the following conditions were observed:—

- (1) After the addition of excess of  $N/5$  bromine, the solution is set aside for complete absorption of the halogen before aeration.
- (2) After aeration, further standing of one hour is required for development of the maximum intensity.
- (3) The concentration of the solution under examination should not exceed 0.003 gram. of alkaloid in 15 c.c. of solution, *i.e.* 0.02 per cent.
- (4) The temperature at which the experiment is carried out should be  $25^{\circ}\text{C}$ .

The details of the colorimetric method evolved for the determination of emetine are as follows:

Fifteen c.c. of the alkaloid, in the form of a solution of a salt, are placed in a 100 c.c. graduated measuring cylinder, and treated with excess of  $N/5$  bromine. The solution is allowed to stand at a temperature of  $25^{\circ}\text{C}$ . for 20 minutes after the addition of the bromine; 25 litres of air are then passed through the solution at the rate of one-half litre per minute, the sides of the cylinder and the aerating tube being washed down after each 5 litres of air are passed, by sucking the liquid up into the bubbling tube and sluicing the cylinder and tube with it. After aeration is complete, the solution is set aside for 1 hour at  $25^{\circ}\text{C}$ . for complete development of the colour. Fifty c.c. of strong alcohol are then added to dissolve any slight precipitate, and the solution is made up to 100 c.c. with distilled water. It is then transferred to a Nessler glass. The colour obtained is compared with those of a series of standards prepared with varying amounts of pure emetine hydrochloride which have been treated with bromine in exactly the manner described, or it can be compared with a single standard in a Duboscq colorimeter. Fairly good results were obtained by using as a standard a  $N/200$  solution of iodine to which 2.5 per cent. of sodium nitroprusside had been added (*i.e.* a mixture of equal parts of  $N/100$  iodine solution and 5 per cent. solution of sodium nitroprusside). In making the comparison in this way 100 c.c. of 50 per cent. (by vol.) alcohol, containing approximately 0.5 gram. of potassium iodide, are put into a Nessler tube and the iodine and nitroprusside solution is added, drop by drop, from a pipette, the liquids being well mixed and compared after each addition, until the colour of the unknown solution is matched. Using this method it was found that:

0.001 gram. of emetine required	1.4 c.c. iodine and nitroprusside solution.
0.002   "       "       "	2.7   "       "       "       "       "
0.003   "       "       "	4.0   "       "       "       "       "

It was also found inadvisable to compare a greater depth of colour than that given by a 2 c.c. addition of iodine and nitroprusside solution, as, if this amount were exceeded, the colours of the standard and the solution diverged somewhat, making comparison difficult. A solution of stronger colour should be diluted with 50 per cent. alcohol until it is within this limit.

**Cephaeline.**—Cephaeline was found to behave in exactly the same manner as emetine, and by comparison with the iodine and nitroprusside standard it was found that the colours developed by the bromine cephaeline compound on aeration were as follows:

0.001	grm. of cephaeline required	1.4	c.c. iodine and nitroprusside solution.
0.002	" " "	2.7	" " " "
0.003	" " "	5.2	" " " "

**Psychotrine.**—Psychotrine was not investigated, but from its chemical structure it is likely that it would behave in a similar manner to emetine and cephaeline.

Based upon the results obtained from the investigation of emetine and cephaeline, the following colorimetric method is recommended for the determination of ipecacuanha alkaloids in such preparations as liquid extract of ipecacuanha, B.P.:

A suitable quantity (1 c.c.) is diluted to 100 c.c. by the addition of distilled water and thoroughly shaken. Ten c.c. are accurately pipetted into a 100 c.c. Nessler glass. This represents approximately 0.002 gm. of ipecacuanha alkaloid. Excess *N/5* bromine (3 c.c.) is added, and the solution set aside for 20 minutes at a temperature of 25° C. Twenty-five litres of air are bubbled through at approximately one-half litre per minute, and the yellow solution is then set aside for 1 hour. The contents are made up to 50 c.c. with distilled water, and 50 c.c. of strong alcohol are then added, and the volume finally adjusted to 100 c.c. with distilled water (A SOLUTION).

Another 10 c.c. of the diluted ipecacuanha solution is transferred to a 100 c.c. Nessler glass and diluted to 50 c.c. with distilled water. Fifty c.c. of strong alcohol are added, and the solution is adjusted to 100 c.c. with distilled water (B SOLUTION).

Another Nessler glass is then taken containing 0.5 gm. of potassium iodide in 100 c.c. of distilled water.\* To this solution is added, drop by drop, *N/200* iodine solution containing 2.5 per cent. sodium nitroprusside, stirring well after each addition. The amount added to match B Solution accurately is noted, and the addition continued until A Solution is also matched. The number of c.c. of iodine and nitroprusside solution required to match A Solution, minus the number

\* NOTE.—To avoid the formation of air bubbles caused by shaking the standard after each addition of iodine and nitroprusside solution it was found more suitable to use one-half per cent. of potassium iodide in water for the standard in place of 50 per cent. alcohol. For very accurate work, a known solution of emetine hydrochloride should be used as a standard, adding *N/5* bromine and aerating in exactly the same manner as with the solution under investigation.

of c.c. required to match B Solution, divided by 1.4, equals the weight of ipecacuanha alkaloid in mgrms. in 0.1 c.c. of the original ipecacuanha, or:

$$\frac{A-B}{1.4} = \text{percentage of ipecacuanha alkaloid in the original strong solution.}$$

**SUMMARY OF WORK ON IPECACUANHA ALKALOIDS.**—(1) Emetine and cephaeline cannot be accurately determined by the titration with sodium thiosulphate of the iodo compound formed by the addition of potassium iodide to the aerated bromine and alkaloid compound.

(2) A reliable means of determination is a modification of this method, using the depth of yellow colour developed by the aeration of the bromine-alkaloidal compound.

(3) Used as a qualitative test, this reaction is sensitive to 1 part of alkaloid in 25,000 parts of solution. In making the test it should be noted that the colour first disappears and then slowly attains a maximum. This reaction is characteristic of the ipecacuanha alkaloids.

(4) A colorimetric method is described for the determination of the ipecacuanha alkaloids in such preparations as liquid extract of ipecacuanha B.P.

#### PART IV. GENERAL APPLICATION OF THE TEST TO SUBSTANCES CONTAINING A PHENOLIC GROUPING.

**PHENOL.**—Pure phenol (6.97 grms.) was dissolved in 1 litre of distilled water. Ten c.c. of this solution were diluted to 100 c.c. with distilled water (1 c.c. = 0.000697 grm. of phenol).

To 1 c.c. of the dilute solution, placed in a 25 c.c. graduated cylinder, were added 12 c.c. of distilled water and 2 c.c. of *N/5* bromine. After 1 minute it was aerated by the passage of 10 litres of air at the rate of 1 litre per minute, at a temperature of 20° C. The solution did not clear on aeration, but contained a flocculent white precipitate. This precipitate tended to creep up the cylinder, but this could be prevented by gently agitating the cylinder during aeration. After aeration was completed, the contents of the cylinder were transferred to a 100 c.c. separator, through a small glass funnel. The cylinder was washed with three 10 c.c. portions of distilled water, and then with 2 c.c. of chloroform, the latter being dropped from a pipette on to the funnel, and finally with 5 c.c. distilled water. Approximately 0.5 grm. of potassium iodide was then added to the solution in the separator and shaken. The phenol-bromine compound goes into solution in the chloroform, and the liberated iodine is titrated with *N/100* thiosulphate, with starch as indicator. If chloroform is not used, the end-point is doubtful, owing to the presence of the flocculent precipitate mentioned. In this way the following results were obtained, the solution being made up to 15 c.c. with distilled water in each case before the bromine was added:

1 c.c. of phenol solution required	1.45 c.c. of <i>N/100</i> thiosulphate.
2 " " " "	2.95 " " "
4 " " " "	5.9 " " "
10 " " " "	14.7 " " "

Therefore, phenol can be determined with accuracy by the method described, 1 c.c. of *N*/100 thiosulphate solution equalling 0.000474 grm. of phenol; i.e. 2 atoms of iodine are liberated on the addition of potassium iodide to the aerated bromine and phenol compound.

In order to ascertain if the bromo-phenol compound was volatile, 50 litres of air were passed through a solution containing 4 c.c. of the diluted phenol treated as described. This required 5.5 c.c. of *N*/100 thiosulphate solution. The compound is, therefore, slightly volatile, but, if the determination is carried out exactly as described, the loss is practically negligible. A correction can be made if desired.

**META-CRESOL.**—Meta-cresol behaves similarly to phenol, 1 c.c. of *N*/100 thiosulphate solution equalling 0.00054 grm. of meta-cresol. Ortho- and para-cresols were not investigated.

**SALICYLIC ACID.**—One grm. of the pure acid was dissolved in 1000 c.c. of distilled water.

On treating different quantities in exactly the manner described for phenol, the following results were obtained:

2 c.c. of salicylic acid solution required				2.8 c.c. of <i>N</i> /100 sodium thiosulphate solution.			
5	"	"	"	7.1	"	"	"
25	"	"	"	35.8	"	"	"

(Where possible, all solutions were made up to 15 c.c. with distilled water before the addition of bromine.)

It was, therefore, concluded that the bromine compound, formed after the addition of excess of bromine and aeration, liberates 2 atoms of iodine from potassium iodide, which can be accurately titrated with thiosulphate solution.

**THYMOL.**—Thymol behaves similarly to phenol. The bromine compound is volatile, and for accurate results rigid conditions must be observed. The thymol (0.283 grm.) was dissolved in 255 c.c. of distilled water and alcohol, 15 c.c. of 90 per cent. alcohol being used (1 c.c. = 0.00111 grm. of thymol). Five c.c. of this solution were diluted to 15 c.c. with distilled water and 4 c.c. of *N*/5 bromine added. The solution was aerated by passing 10 litres of air; potassium iodide was then added, and the titration was carried out as for phenol, 7.15 c.c. of *N*/100 thiosulphate being required. Another 5 c.c. treated as above described, but with 50 litres of air used for aeration, required only 6.15 c.c. of *N*/100 thiosulphate solution. A correction of 0.2 c.c. should, therefore, be made for the volatility of the bromine compound, 7.15 c.c. thus becoming 7.35 c.c. With this correction, 2 atoms of iodine are liberated from 1 molecule of the aerated thymol and bromine compound on the addition of potassium iodide.

Other common substances, such as benzoic acid, tartaric acid, citric acid, etc., when tested gave negative results. It would, therefore, appear that the reaction

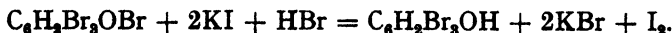
described is characteristic of a phenolic grouping, and, if conditions are modified to suit the particular substance under investigation, it should be possible to determine quantitatively most of these substances in this manner.

GENERAL CONCLUSIONS.—Taking phenol as the simplest substance investigated, the chemistry of the reaction would appear to be:

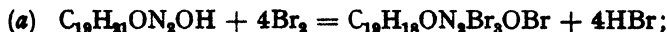
- (a) On the addition of excess bromine and aeration



- (b) On the addition of potassium iodide



The liberated iodine is titrated with thiosulphate, with starch solution as indicator. Of the more complicated alkaloidal substances, cupreine is known to contain a phenolic grouping. It is, therefore, reasonable to suppose that the reaction would proceed as follows:



Quinine, however, does not contain a phenolic grouping. A. Christensen (*Ber. Deut. Pharm. Ges.*, 1916, 25, 256) describes the progressive action of chlorine on quinine. He gives a base,  $\text{C}_{19}\text{H}_{21}\text{O}_3\text{N}_2\text{Cl}_3$ , which is obtained by the action of 3 molecules of chlorine on 1 molecule of the alkaloid. This base contains one active chlorine atom capable of displacing iodine from potassium iodide and so probably containing the grouping— $\text{CO.CCl}_2$ —in the quinoline nucleus. By analogy, the base formed on the addition of bromine, and aeration, to a quinine solution would be  $\text{C}_{19}\text{H}_{21}\text{O}_3\text{N}_2\text{Br}_3$ , which would contain the grouping— $\text{CO.CBr}_2$ —also in the quinoline nucleus. This group by undergoing conversion into  $\text{C(OH):CBr}$ —would liberate two equivalents of iodine on the addition of potassium iodide, although one molecule of bromine only is active in the reaction. Thus in the case of quinine the reaction, as described, would proceed along phenolic lines, although no phenolic grouping is present originally in the alkaloid. Comanducci (*J. Chem. Soc.*, Abst., 1910, i, 581) also supports the hypothesis of a phenolic grouping being active in the thalleioquin reaction. With regard to the other alkaloids investigated, morphine is known to contain a phenolic group, and codeine, on treatment with a halogen, would probably undergo an intra-molecular rearrangement similar to that of quinine.

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## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### THE APPLICATION OF PIPERIDINIUM PIPERIDYL-DITHIOFORMATE TO THE COLORIMETRIC DETERMINATION OF COPPER.

HAVING found that, on the addition of an alcoholic solution of piperidinium piperidyl-dithioformate to a solution of a copper salt, a yellowish-brown coloration is produced, I made this the basis of a colorimetric method of determining copper in small amounts. This colour is very stable and is not affected by the presence of a slight excess of acid or alkali. Cadmium, mercury or bismuth cause no interference; iron, however, interferes to a pronounced extent, and must be removed. The maximum sensitivity of the test, when carried out in ordinary 50 c.c. colorimeter glasses, is of the order of 0.00001 grm. of copper. The following procedure is recommended:—Dissolve the sample in hydrochloric acid, dilute the solution with water so that the acid concentration does not exceed 15 per cent. by volume, heat to boiling, add a few drops of nitric acid, and pass a current of hydrogen sulphide through the solution until it is cold. Allow the precipitate to settle, filter by suction through a sintered glass crucible (Jena), wash with water acidified with acetic acid and saturated with hydrogen sulphide, until no more iron can be detected in the filtrate, place the crucible on a glass (or silica) triangle over a small crystallising dish standing on a glass water-bath, and add fuming nitric acid to the crucible. When no more liquid remains in the crucible, wash it with water and allow it to remain on the water-bath until the solution containing the copper has evaporated. Place the crystallising dish on a hot plate until no more acid fumes are given off, taking care not to decompose the nitrate. Take up the residue with water. This procedure has the following advantages:—(1) Complete separation from the metals of the 3rd group; (2) separation from mercury salts; (3) final solution practically neutral. Care must be taken, however, that the copper sulphide precipitate does not remain in contact with the air, and copper contamination must be excluded, *e.g.* by placing a glass plate over the crucible when filtering and dissolving. A very small amount of copper (a few thousandths of 1 mgrm.) can be precipitated and recovered by this method.

**DETERMINATION OF THE COPPER.**—The final solution, containing cupric ions, is transferred to a graduated glass-stoppered flask. If the colour of copper sulphide in the crucible has been noted previously, it is easy after several analyses to estimate roughly the copper present. This approximate estimation indicates the size of the flask which it is desirable to use. Aliquot portions of the neutral solution are compared with a standard solution of 0.393 grm. of pure (A.R.) copper sulphate in 1 litre (1 c.c. = 0.1 mgrm. of copper).

The colour comparison is made by adding 1 c.c. of 0.1 per cent. alcoholic solution of piperidinium piperidyl-dithioformate to the solution under examination and to the standard; it is most accurate with amounts of copper ranging from 0.00001 to 0.00008 grm. If desired, the piperidinium piperidyl-dithioformate may

be added first, and portions of the standard run in from a burette until the desired match is obtained. The colour is obtained immediately and is quite stable.

The following figures were obtained by using a Lovibond Tintometer (British Drug Houses) to match the colour.

Copper added mgrm.	Tintometer readings	
	Red	Yellow
0.002	0.2	0.5
0.003	0.2	0.7
0.004	0.4	0.7
0.005	0.7	0.8
0.006	0.2	1.8
0.007	0.4	1.9
0.008	0.7	1.7
0.009	0.8	2.4
0.010	1.3	4.0
0.020	1.8	7.0
0.030	2.6	8.9
0.040	3.6	11.0
0.050	4.0	15.0
0.060	5.0	18.0
0.070	5.9	19.0
0.080	6.6	19.1
0.090	5.9	21.0
0.100	7.8	24.0

North daylight was used for the comparison, and the solution was adjusted to 7 c.c. in a glass cell with internal dimensions (38×20×13 mm.).

The organic matter may be oxidised by ashing the substance in an electric muffle as follows: A large sample in a silica dish is placed on a hot plate until it is charred, and then in an electric muffle at a temperature not exceeding 400° C. After 30 minutes the dish is removed and allowed to cool, and its contents are treated with fuming nitric acid and evaporated to dryness on a hot plate. Sometimes the oxidation is complete after this treatment. If only a small quantity of organic matter remains, more nitric acid is added, and the mixture is again evaporated to dryness. With larger amounts the dish must be replaced for 30 minutes in the muffle and, after cooling, more nitric acid must be added. As a rule, all the organic matter is completely oxidised by this treatment, which should be repeated as often as necessary. Gebhardt and Sommer found that ashing in the muffle is liable to give low values if the temperature of the muffle is too high.

I wish to thank the Lovibond Tintometer Company for the loan of the instrument used in the above determination.

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## Standing Committee on Uniformity of Analytical Methods.

### UNSAPONIFIABLE MATTER IN FATS AND UNSAPONIFIED FAT IN SOAPS.

THE Standing Committee on Uniformity of Analytical Methods has appointed a Sub-Committee to formulate methods for the determination of these substances. The Members of the Sub-Committee are Prof. T. P. Hilditch, D.Sc., F.I.C. (Chairman), and Messrs. E. R. Bolton, F.I.C., L. V. Cocks, F.I.C. (Hon. Sec.), F. R. Ennos, B.Sc., F.I.C., N. Evers, B.Sc., F.I.C., L. A. Jordan, D.Sc., F.I.C., and W. H. Simmons, B.Sc., F.I.C.

## Eighth Report of the Essential Oil Sub-Committee to the Standing Committee on Uniformity of Analytical Methods.

### THE DETERMINATION OF CINEOLE IN ESSENTIAL OILS.

#### SECOND REPORT

THE Sub-Committee makes the following recommendations in regard to the determination of cineole in essential oils other than cajuput and eucalyptus oils.

#### (II) CAMPHOR OIL.

The ortho-cresol method, which was the subject of the Sub-Committee's first Report (ANALYST, 1927, **52**, 276-9), has been shown to be reasonably accurate when applied to camphor oil. As this oil usually contains less than 50 per cent. of cineole, the modification of the method mentioned on the second page (*ibid.*, p. 277) of the Report must be used.

This modification involves either enriching the oil by the addition of an equal weight of pure cineole before testing; or adding to the mixture of 3 grms. of the oil and 2.1 grms. of ortho-cresol an equal weight (5.1 grms.) of the pure recrystallised ortho-cresol compound, and then carrying out the test in the usual manner and making the necessary correction.

In the case of light camphor oil, *i.e.* one distilling below 200° C., the modified test is carried out directly on the oil. When a camphor oil contains high-boiling constituents, such as camphor and safrole, it is necessary to distil the oil through a fractionating column and carry out the test on the fraction boiling below 200° C. The cineole content of the original oil is then obtained by calculation.

The accuracy of the table of freezing points published in the first Report, when applied to camphor oil, has been confirmed by the members of this Sub-Committee. For this purpose mixtures of known cineole content were prepared by mixing pure cineole with a diluent prepared as follows:—A mixture of American turpentine, camphor and safrole was distilled through a fractionating column, and the fraction distilling up to 200° C. was collected. This was taken as representing the non-cineole portion of light camphor oil and was used for diluting the cineole.

Mixtures containing 50, 60, 66·7, 70, and 75 per cent. of cineole were prepared, such mixtures representing camphor oil containing, 0, 20, 33·3, 40 and 50 per cent. of cineole enriched with an equal weight of pure cineole.

The results obtained are shown in the following table:

SUMMARY OF RESULTS OF THE DETERMINATION OF CINEOLE IN ARTIFICIAL MIXTURES REPRESENTING CAMPHOR OIL ENRICHED WITH AN EQUAL WEIGHT OF CINEOLE.

Sub-Committee Member.	ACTUAL CINEOLE CONTENT.									
	50 Per cent.		60 Per cent.		66·7 Per cent.		70 Per cent.		75 Per cent.	
	F.Pt. = Per cent. °C.	Per cent. cineole.	F.Pt. = Per cent. °C.	Per cent. cineole.	F.Pt. = Per cent. °C.	Per cent. cineole.	F.Pt. = Per cent. °C.	Per cent. cineole.	F.Pt. = Per cent. °C.	Per cent. cineole.
No. 1	27·0	49·5	35·4	60·4	39·8	66·5	42·1	70·7	44·8	75·7
No. 2	27·6	50·3	35·6	60·7	40·2	67·2	42·0	70·5	44·8	75·7
No. 3	27·5 27·9	50·2 50·7	35·7	60·8						
No. 4	28	50·8	36	61·2	40·6	67·8	42·3	71·1	45·2	76·5
No. 5	27·8	50·5	35·8	61·0	50·3	67·3	42·2	71·0	45·0	76·1
No. 6	27·1 27·4	49·6 50·0	35·2 35·4	60·2 60·4	39·8 39·8	66·5 66·5	41·8 41·8	70·1 70·1	44·8 44·8	75·7 75·7
No. 7	27·2	49·8	35	60	40·05	67·0	41·8	70·2	44·7	75·6
No. 8	27·4	50	34·7	59·5	39·5	66·0	41·6	69·7	44·5	75·2
Mean		50·1		60·5		66·9		70·4		75·8
Variation from mean		-0·6 +0·7		-0·9 +0·4		-0·8 +1·0		-0·6 +0·8		-0·5 +0·8
Maximum variation	1·0°	1·3 per cent.	1·0°	1·3 per cent.	1·1°	1·8 per cent.	0·7°	1·4 per cent.	0·7°	1·3 per cent.

These results show that the experimental error between different analysts does not exceed  $\pm 1$  per cent., but, as they require correcting for the added cineole, the limit of experimental error must be stated as  $\pm 2$  per cent.

### (III) OTHER CINEOLE-CONTAINING OILS.

The presence of alcohols, esters, aldehydes and ketones in quantity in cineole-containing oils has been shown to raise the freezing-point of the ortho-cresol compound. The method, therefore, indicates a higher result than the actual content. No means has been found for carrying out accurate determination under these conditions, but the Sub-Committee is of opinion that the "apparent" cineole content shown by the ortho-cresol method has a considerable value. Fractions of eucalyptus oil are frequently used as adulterants in rosemary oil, and the apparent cineole content is a useful figure in the examination of such oils. The Sub-Committee recommends that the term "Apparent Cineole Content by Ortho-cresol" should be used in connection with the oils of rosemary, spike, and sage.

(Signed),

John Allan (Chairman), C. T. Bennett, S. W. Bradley, E. Theodore Brewis, L. E. Campbell, Thos. H. Durrans, T. W. Harrison, Ernest J. Parry, C. Edward Sage, W. H. Simmons. T. Tusting Cocking (Hon. Sec.).

June 17th, 1931.

## Notes from the Reports of Public Analysts.

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF BIRMINGHAM.

#### REPORT OF THE CITY ANALYST FOR THE SECOND QUARTER, 1931.

THE number of food and drug samples examined during the quarter was 1230, of which 1181 were informal samples; of these, 142 were adulterated or incorrect.

**VINEGAR.**—Three samples were described as "Non-brewed Vinegar" on the label, and underneath in smaller type, "Suitable for Table Use." This appears to be a contradiction in terms, since table vinegar should consist of malt vinegar; actually, all three samples consisted of artificial vinegar. No action, however, was taken.

**CRYSTAL MINTS.**—Two samples contained, respectively, 840 and 460 parts of sulphur dioxide per million. The manufacturers of both were cautioned and agreed to take immediate steps to see that their products complied with the regulations.

Glucose syrup, according to the first Schedule of the Preservatives in Food Regulations, is allowed a maximum of 450 parts per million, and cane sugar a maximum of 70 parts of sulphur dioxide per million, and calculations from the composition of these mints showed that neither should have contained more than about 150 or 160 parts per million.

**BEEF SUET.**—The label of this sample stated that it would go half as far again as raw suet, and instructions were given for the use of one-third less than one would use of raw suet or lard. As the sample contained only 84 per cent. of fat, as against about 95 per cent. for raw suet and 100 per cent. for lard, this was, by implication, a false label, and the makers were cautioned.

**"FRUIT DRINKS."**—The labels of these samples described them as "Fruit Drinks," and the advertisements connected with them implied that fruit juice was used in their manufacture. As a matter of fact, the only connection with fruit was that oil of lemon or oil of orange and citric acid were present in very small quantities. No genuine fruit juice was present. The makers were approached and have agreed initially to withdraw the term "Fruit Drink," as applied to their product. Correspondence is still in progress with the firm, and it is hoped that the position will, in due course, be entirely satisfactory.

**DEVONSHIRE CREAM CHEESE.**—This article was advertised in the vendor's shop under this name, but the sample contained only 56 per cent. of fat calculated on the dry solids, whereas genuine cream cheese should contain at least 80 per cent. calculated in this way. The article was obviously ordinary whole-milk cheese. The vendor apologised, saying that he acted in ignorance and immediately withdrew the offending label.

H. H. BAGNALL.

## METROPOLITAN BOROUGH OF STEPNEY.

## ANNUAL REPORT OF THE BOROUGH ANALYST FOR THE YEAR 1930.

OF the 1602 samples submitted, 1532 were foods and drugs, 948 being formal, 570 informal, and 14 "appeal to the cow" samples. Thirty-five samples were adulterated.

**THE WARRANTY DEFENCE.**—Proceedings were taken against the vendor of a sample of milk which was 8.3 per cent. deficient in fat. Three samples taken from the wholesaler were genuine, containing 3.50, 3.65 and 3.70 per cent. of fat, respectively, but it was stated for the defence that, although these samples were genuine, the results were of no importance, and that the samples were taken merely to comply with the regulations, so that the retailer might be entitled to plead a warranty defence. The warranty was produced, and the summons was dismissed, that being the sixth occasion upon which summonses against the vendor had been dismissed after the production of a warranty. If such a defence is to hold good in cases where the wholesaler's sample is genuine, there is no improvement on the old warranty conditions; neither party can be convicted, and the position remains as before.

Some defendants apparently do not bother to obtain a warranty. One wholesaler in the Borough refuses to give a warranty, but will pay the fines or costs should proceedings be taken against the vendor.

**FACED PEARL BARLEY.**—One sample of seven examined was adulterated with 0.65 per cent. of talc powder. It is highly important that pearl barley should be free from contamination of this kind, owing to its extensive use in the preparation of barley water for invalids and children.

**PEPPER MIXTURE.**—Fifteen samples of pepper were examined. One sample, which contained 50 per cent. of rice starch, bore the label: "This compound is sold as a mixed article, and is warranted to be of choice quality. Sale of Food and Drugs Act."

**DIABETIC FLOUR.**—A sample was taken formally, following the analysis of the same flour (Diabetic Flour, No. 1) for a private purchaser. It consisted of ordinary wheat flour in which the protein had been increased by about 10 per cent. (on the flour), the normal amount of starch being, in consequence, reduced to that extent. There could be no great difference in effect between taking this flour or a little less of an ordinary flour. It was sold in 6 lb. bags at 6s. 6d. per bag.

The sample was condemned as being "sold to the prejudice of the purchaser" and "not of the nature, substance and quality demanded." On the instruction of the Public Health Committee the vendor was cautioned. He stated that the flour was sold exactly as it was received from the millers, and that the millers accepted responsibility for the article. The millers stated that they published a booklet containing a warning that no diabetic patients should select their diet without first consulting a doctor (this booklet was not given with the flour), and that they sold three kinds of flour: No. 1, for mild cases; No. 2, for severe cases; and No. 3, for acute cases. They agreed to place an analysis of the contents in each bag.

**ROOT GINGER.**—A sample of root ginger contained 95 parts of sulphur dioxide per million. An article of food may contain preservative if it is to be used in the preparation of one of the foods in which preservative is permitted. If the root is used in the preparation of ginger wines (alcoholic and non-alcoholic), preservative

is permitted; but if the root is ground for use in cakes and puddings, preservative is not permitted.

**METALS IN TINNED TOMATOES.**—Two samples of tinned tomatoes were unsatisfactory owing to the presence of excessive amounts of tin and copper. The samples contained respectively 0·7 grain copper per pound and 2·7 grains tin per pound, and 0·6 grain copper per pound and 2·6 grains tin per pound.

Copper, when present as a metallic colouring matter, is prohibited; there is, however, no legal standard controlling the presence of copper otherwise in food.

**METHYLENE BLUE IN TINNED PEAS.**—A sample of tinned peas was examined for metallic contamination and for the presence of a dye. Complaints had been made of the effects produced after the peas had been eaten. Owing to the discontinuance of the use of copper salts for the production of a green colour in peas, other methods are now being used. In this case the peas were found to have been coloured with methylene blue. When this dye is taken internally it has the effect of colouring the urine green or blue according to the amount of dye present. This dye is not, however, included in the list of dyes prohibited for use in colouring food in this country, and its use, therefore, is permissible. Dyes, however, when combined with specified metals, are prohibited. Methylene blue occurs commercially in combination with zinc. As the zinc combined with the small amount of dye would be extremely small, it is not possible to say whether the methylene blue was originally combined with zinc or not, as appreciable amounts of zinc may occur naturally in tinned foods or accidentally from the soldering flux.

DOUGLAS HENVILLE.

## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### DISTINCTION BETWEEN POTTED SALMON AND SALMON PASTE.

ON May 5th a shop-keeper was summoned at Leeds for selling adulterated potted salmon.

The certificate of the Public Analyst (Mr. C. H. Manley) was to the effect that the sample contained 80 per cent. of salmon and 20 per cent. of a starchy "filler."

Mr. H. R. McDowell, prosecuting, said that the substance known as potted salmon should contain only pure salmon treated with melted butter, and a little seasoning. The substance which was widely sold should be known as salmon paste. It was only fair to the defendant, however, to say that only the commercial rate for salmon paste was charged and not that for potted salmon. The Health Authorities considered that there was a very big volume of meat food substances sold in Leeds under the designation of potted salmon, or potted meat, whereas, in fact, they contained a proportion of starchy substance and should be described as salmon paste or meat paste.

The summons was dismissed on payment of costs.

## Ministry of Health.

### TWELFTH ANNUAL REPORT, THE YEAR ENDING MARCH, 1931, WITH ABSTRACT OF REPORTS OF PUBLIC ANALYSTS.\*

**SALE OF FOOD AND DRUGS ACT.**—Of a total of 136,515 samples examined (an increase of 2931 over 1929, *cf.* ANALYST, 1931, 110), 6496 (or 4·8 per cent.) were reported against, and in 519 instances the Public Health Preservatives Regulations were reported to have been contravened. One sample sold as preservative powder consisted of ground rice starch.

**Spirits, Brandy, etc.**—Two hundred and five samples of spirits were below the standard of 35 degrees under proof; traces of tin were present in three samples of rum; lead was present in two samples of beer, and a coal tar disinfectant in two samples of stout (probably due to insufficient washing of the bottle).

**Coffee, Cocoa and Tea.**—Most of the samples of coffee reported against contained chicory; arrowroot was present in one cocoa; five samples of tea contained iron filings, and four others extraneous mineral matter.

**Non-Alcoholic Wines.**—Two samples contained, respectively, 14·4 and 14·3 per cent. of alcohol.

**Other Foods.**—Of 179 samples of bread-and-butter sold as such, 35 were made with margarine or a mixture of margarine and butter. Arsenic was present in 18 samples of sweets reported against, and 38 other samples contained excess of sulphur dioxide. A "rum and butter" toffee contained no butter, and the "rum" was a synthetic flavour; other "butter" sweets contained little or no butter. Copper was present in two samples of peas and in one of cider, and in the "gold tips" of two samples of sweetmeat cigarettes, and lead was reported in five samples of cider, one of cake, one of beer and one of lemonade. Tin was present in 53 samples of tinned foodstuffs, including fruit, vegetables, fish, soup, and black treacle, and one sample of corned beef and one of a sauce contained zinc. Contamination of cheeses wrapped in tin foil is being dealt with by the manufacturers and others in order to reduce it to the lowest possible limit, but 19 samples were reported as unsatisfactory, owing to tin being present in varying amounts up to 8·33 grms. per lb.

**MILK AND PARATYPHOID FEVER.**—Inquiries into an outbreak of paratyphoid fever, involving some 300 persons and resulting in seven deaths, established strong *prima-facie* evidence against milk, as all the primary cases had consumed raw milk from a farm where an employee was found to be suffering from a mild attack of the fever. The milk supply was temporarily stopped, a new infection-free staff of milkers engaged, and, after thorough disinfection of all the utensils, the milk was pasteurised before distribution to retailers.

D. G. H.

### REPORT OF THE CHIEF INSPECTOR OF FACTORIES AND WORKSHOPS FOR THE YEAR 1930. INDUSTRIAL DISEASES.†

AMONG the subjects dealt with in the Report are the following:

**RESPIRATORS.**—An investigation into the efficiency of various types of respirators is being made by the Department of Scientific and Industrial Research.

\* Obtainable at Adastral House, Kingsway, W.C.2. Price 5s. net.

† Obtainable at Adastral House, Kingsway, W.C. 2. Price 2s. 6d. net.



**LEAD POISONING.**—The number of notified cases of lead poisoning was 265 (as compared with 244 for 1929) with 32 deaths, 10 for the pottery industry, and 13 in the painting of buildings. The Lead Paint (Protection Against Poisoning) Regulations of 1927 cannot be expected to be felt for some years.

**ARSENICAL POISONING.**—The one reported case was that of a man of 61, employed for 46 years in the manufacture of sheep dip, who, 10 years previously, had had an epithelioma removed from the right shoulder, and five years later another from the neck, and now had one on the left upper arm.

**ANILINE POISONING.**—The 24 (26 in 1929) cases of aniline poisoning reported (no deaths) included 10 due to inhalation of the vapour from 5-chlor-ortho-toluidine emitted from the paste which had been dried in a vacuum oven, with an abnormally high outside temperature. Headache, drowsiness and conjunctival or nasal irritation were followed by more serious effects, causing 10 of 13 men to cease work. Making intermediates accounted for 8 cases; aniline colours, etc. for 3; aniline black dyeing for 2, and handling aniline residue for one. An inquiry into ill-health in boot factories arising from the use of stains containing aniline oil has shown that nitrobenzene, in the proportion of 1: 50, has been used for three years as a solvent for certain dyes made in imitation of foreign preparations, and that more black spirit-stain than formerly is being used to cover defects of poor leather. Any cases of ill-health appear to be among wearers of recently dyed shoes and not among the operatives handling the dyes.

**TETRACHLOROETHANE POISONING.**—Several cases of jaundice associated with plant for degreasing wool were due to tetrachloroethane, but, if the concentration of this chemical in the air is below about 5 per cent., no permanent ill-effects appear to arise. Other less toxic solvents, producing, so far as is known, no chronic effects, may be used, but in sufficient concentration they have anaesthetic properties.

**DERMATITIS.**—Voluntarily reported cases of dermatitis numbered 789, and the increase is attributed to the greater importance assigned to the condition. Dermatitis cases may be reduced to a small proportion by strict adherence to the advice in the official pamphlets and placards. The method of cleansing the hands and arms after work was responsible for a large number of cases, and it is recommended that, if possible, a film of ointment should be applied to the skin before handling staining preparations; cleansing agents should be as weak as efficiency will permit, and thorough washing and a light application of ointment should follow the removal of stains from the skin. An alkaline antiseptic wash, such as liq. chlorinated soda with boric acid (B.P.C.), will prevent oil dermatitis.

**OTHER GASEOUS POISONS.**—Hydrogen sulphide poisoning, as the result of accidents, was reported in 5 cases. There were 5 cases of chlorine poisoning and 2 of ammonia poisoning. Benzole fumes poisoned 6 persons, one fatally.

**NICOTINE POISONING.**—Two oz. of concentrated nicotine were accidentally spilt on a girl's arm. The arm was washed with hot water, but in half-an-hour the girl was sick and collapsed. The clothing was removed and the skin was at once cleansed, and, after artificial respiration and cardiac stimulation, recovery took place.

**CELLULOSE LACQUER RISKS.**—Examination of the air of a room being decorated by spraying low-viscosity cellulose paints containing (1) 30 to 40 parts by weight of toluole, (2) 60 per cent. of xylol, with benzole as impurity, showed that 3 parts of xylol or toluole per 10,000 (in terms of benzole) is the maximum quantity which can be safely inhaled over long periods. In none of the 7 men engaged on the spraying could benzole poisoning be diagnosed.

D. G. H.

# International Register of Spas and Medicinal Waters.

(See also the Review on p. 776.)

IN 1927 the Council of the International Society of Medical Hydrology appointed an International Standard Measurement Committee to formulate terms of expression to be known as "International Standard Measurements," and it is proposed that the letters "I.S.M." should henceforth be used to designate analytical and other data obtained in accordance with the prescribed methods.

## I. INTERNATIONAL INFORMATION.

**CLASSIFICATION OF WATERS.**—A standard classification of waters according to their chemical, physical and medical characters is recommended, and examples to illustrate the style of reporting are given.

### A. The Chemical Classification should be expressed as follows:

- (a) Its composition in terms of its one or more dominant or most active ions; for example, chloride, sulphide, sulphate, calcium, ferrous iron, arsenic, etc. When desired, these may be followed by brackets enclosing symbols of other active ions in the order of their medicinal importance.
- (b) Its *concentration* (ionic concentration) in terms of millinormality ( $N/1000$ ).
- (c) The *reaction* (hydrogen ion concentration) should be given in terms of pH at 20° C.; or at other temperature, if stated.

### B. The Physical Classification according to

- (i) *Temperature* (at the source):—(a) cold, below 20° C.; (b) thermal, 20° to 37° C.; (c) hyperthermal, above 37° C.
- (ii) *Radioactivity*, (a) of the water itself, or (b) of the contained gas.
- (iii) *Tonicity*, which may be expressed by the terms *hypotonic*, *isotonic*, *hypertonic*, in comparison with body fluids.

C. **The Medicinal Classification** should be based on analytical data and clinical experience, and be expressed in not more than ten words, using (a) *for their internal action*, aperient, diuretic, solvent, alterative, tonic, etc., and (b) *for their external action*, temperature effects, sedative, stimulant, counter-irritant, etc. *Disorders* for which the water is particularly indicated should then be named.

**CALCULATION OF SALINES.**—The analysis of a water gives the composition directly in terms of *Ions*, and from these data "Salines" may be derived by calculation. The letters "I.S.M." should be shown at the head of the saline table, whenever the salines have been calculated in accordance with the prescribed formula. *It is particularly requested that salines shall NOT be given if calculated according to any other formula; or, if so, that the fact be very clearly indicated. Reporting salines is entirely optional; it is not recommended.*

**Formula for Calculating Salines.**—Calculate (a) bromides, iodides, fluorides, sulphides (as hydrosulphide, NaHS), phosphates ( $\text{Na}_2\text{HPO}_4$ ), arsenites ( $\text{Na}_2\text{HASO}_3$ ) and other anions present in only small amount as sodium salts; (b) Rubidium, caesium and, in non-alkaline waters, lithium and other cations present in only small amount, as chlorides; (c) in alkaline water, lithium, strontium, barium, zinc, lead, tin, manganese, aluminium and iron as hydrocarbonates; (d) in acid waters (pH < 4), iron and aluminium as sulphates; (e) the major ions in the following order, after deducting from the total quantity of an ion present the sum of the quantities used for the foregoing ions.

Anions	$\text{NO}_3'$	$\text{Cl}'$	$\text{SO}_4'$	$\text{HCO}_3'$	$\text{CO}_3'$	$\text{OH}'$
Cations	$\text{K}^{\cdot}$	$\text{Na}^{\cdot}$	$\text{Ca}^{++}$	$\text{Mg}^{++}$		

(f) Silicon, titanium and boron as directed *infra*. In alkaline waters, the concentration of the anions of these weak acids should be calculated by means of the dissociation constant and the pH value.

Occasionally some slight departure from the formula is desirable, as when an ion is present in unusually large or unusually small proportion, or, for example, when sulphide occurs in a

normal sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) water it is better reported as "sulphide,"  $\text{Na}_2\text{S}$ . When aluminium and phosphate ions are associated in appreciable amounts, they may be calculated in that form of aluminium phosphate which is in agreement with the pH value of the water.

**NATIONAL REGISTERS OF WATERS.**—At the annual meeting at Budapest, in 1929, the Society published specimen pages of a National Register, giving data relative to the spa, as well as those relative to the water, and, the style having been generally approved, it provides the model on which the Register now adopted is compiled.

## II. DATA CONCERNING THE WATERS THEMSELVES.

(i) The analysis, whether expressed in ions or salines, shall be in terms of parts per million; either as mgrms. per litre or mgrms. per kilo.

(ii) The quantities of ions, also "salines," when given shall be given to one decimal place only, or, when the quantity is less than ten, to two significant figures, e.g. 357.6, 6.4, 0.18, 0.0027.

(iii) The analysis of a water shall always be expressed in terms of ions, whether its interpretation in other terms be given or not.

(iv) The analysis may also be expressed as "salines" provided these are computed by the arbitrary method of calculation which the Society has approved for international adoption.

(v) The analysis, whether given as ions or as salines, shall be expressed in terms of "millinormality" ( $N/1000$ ).

(vi) The specific gravity shall be determined by comparison with distilled water at the same temperature, preferably between  $15^\circ$  and  $20^\circ$  C.

(vii) Analytical tables shall be printed in four columns, in the following order:—(a) The name of the ion or saline. (b) Its concentration in the terms prescribed, marked "I.S.M." (c) The same, in terms customary in the country where the water originates, in those cases where these differ from the international terms, marked "National." (d) The same in terms of millinormality, marked " $N/1000$ ."

(viii) Gases shall be determined in terms of the number of c.c. at normal temperature and pressure ( $0^\circ$  C. and 760 mm.) contained in a litre of the water.

*Spectroscopic Analyses* under modern conditions, exploring both the visible and ultra-violet regions of the spectrum, should be made whenever possible. Elements recognised and estimated by spectroscopic methods should be indicated in the tables of ions by "Sp." against the quantity found; for example,  $\text{HBO}_3$  trace (Sp.); Cr. 0.0015 (Sp.). When necessary, a chemical result may be distinguished by "Ch."

THE MINERAL CONSTITUENTS AND GASES should be reported in accordance with the foregoing resolutions and with the directions for printing the data. The following details should also be observed:—(i) The ions should be arranged in the following order, which is convenient and is an approximation to the periodic classification; the most commonly occurring are:

(a) cations :  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{Ba}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Sn}^{++}$ ,  $\text{Pb}^{++}$ ,  $\text{Bi}^+$ ,  $\text{Cr}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{Fe}^{++}$ ,  $\text{Fe}^{+++}$ ,  $\text{Al}^{+++}$ .

(b) anions :  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{HS}^-$ ,  $\text{NO}_3^-$ ,  $\text{IO}_3^-$ ,  $\text{SO}_4^{--}$ ,  $\text{PO}_4^{---}$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{--}$ ,  $\text{OH}^-$ .

(ii) Boron, Silicon and Titanium should be given in terms of "Boric Acid— $\text{HBO}_3$ "; "Silicic Acid— $\text{H}_2\text{SiO}_3$ "; "Titanic Acid— $\text{H}_2\text{TiO}_3$ " respectively, after the summation of the anions.

(iii) The total sum of the items determined should then be given.

(iv) Total Solids should, when the water after boiling, and cooling, gives an alkaline reaction with phenolphthalein, be determined by drying to constant weight at about  $180^\circ$  C. When the water does not so react, the total solids should be determined by the sodium carbonate method. This is followed by adding a small known quantity of sodium carbonate to the water before evaporating, then drying to constant weight at about  $180^\circ$  C., and deducting from the weight so found the weight of the sodium carbonate added. The quantity of water employed may conveniently be such as will yield about one grm. of total solids.

(v) Ions sought but not found should be expressed by chemical symbols in small type in a single line; followed by "sp." = spectroscopically, "ch." = chemically. Such negative information is valuable *per se*, but also it is an indication of the completeness of the analysis.

**PHYSICAL AND OTHER PROPERTIES OF THE WATER.**—Under this heading may be included any suitable data which are satisfactory; for example:—(i) *Specific gravity*. (ii) *Turbidity or Colloidal properties* of the water as it issues from the source, or so many hours after collection.

(iii) *Degree of Ionisation*. The basis on which this is calculated should be given; for example, the *Cryoscopic Constant*, the *Ebullioscopic Constant*, the *Electrical Conductivity*, etc., giving the figures for each basis quoted. (iv) *Hydrogen-Ion concentration*. The means adopted for its determination should be indicated. (v) *Radioactivity*. This should be given in terms of radons or micromillicuries per litre. (vi) *Describe any changes* that take place in the physical and chemical condition of the water during a period of hours or days after it leaves the source.

### III. DATA RELATIVE TO THE SPA.

Measurements should be given in the usual metric terms, but the same measurements in other terms may be added in brackets.

CLIMATE should be described with regard to (a) *Character*: whether "tonic," "sedative," "sheltered"; (b) *Temperature*: (i) during the year, (ii) during the season; (c) *Rainfall*: (i) during the year in cm.; (ii) during the season in cm.; (d) *Atmospheric humidity*; (e) *Prevailing winds*.

Other data required include *Character of the surroundings* and the *Geology*: (a) of the district; (b) of the spring. "Description" of the springs. Details of other springs in the neighbourhood.

### IV. STYLE AND COMPOSITION OF "THE REGISTER."

The sections comprise (i) "International Information"; (ii) "National Information"; (iii) The "data" relative to the spas; (iv) Appendix of unofficial information.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Detection of Certain Types of Heated Milk.** S. Rothenfusser. (*Z. Unters. Lebensm.*, 1931, 62, 210-214.)—Various processes for heating milk are discussed with special reference to the author's method of detecting heated milk (*ANALYST*, 1930, 55, 758). The biological, bacteriological and chemical importance of processes which involve heating at about 79 to 85° C. is emphasised, and an oxidation-reduction reaction for such milk, suitable for routine work, is proposed. Di-*p*-diamino-diphenyl or (preferably) di-*p*-diamino-diphenylamine sulphate is used as a chromogen, since normally it is stable, but, on oxidation, gives a blue quinonoid compound (indamine). It is mixed with an organic acid (preferably tartaric acid) which has no water of crystallisation, slight acidity being required to promote the reaction. The oxygen carrier is magnesium perborate, which is stable and provides the correct degree of buffering in the milk. Since the chromogen is only sparingly soluble in water or organic solvents, the solid reagents are mixed and inserted in the top of a vertical glass tube containing an axial rod or plunger. The bottom of the tube dips into 5 c.c. of milk in a 10 c.c. tube, and the reagent is added at will by manipulating the central rod. Milk which has been heated at 79° to 80° C. retains its colour, but if a lower temperature has been used a blue colour results, whilst heating at 77° C. or for a brief period (e.g. 30 seconds) at 79° C. results in a slow reaction. The test should not take longer than 1 minute. J. G.

**Suggested Method for the Determination of the Dextrinolytic Activity of Malt.** L. Fletcher and J. B. Westwood. (*J. Inst. Brewing*, 1931, 37, 470-478.)—The results of preliminary experiments indicate that J. L. Baker's

$\alpha$ -amylodextrin (*J. Chem. Soc.*, 1902, 81, 1177) is a suitable substrate for determining the dextrinolytic activity of aqueous malt extracts. The early stages of the action of malt extract on this dextrin represent substantially the work of a single enzyme, dextrinase, or of two enzymes acting with equal velocities, since, up to the production of maltose corresponding with about R15, the velocity constant of the reaction remains practically constant. The procedure for the test is as follows: 30 grms. of the air-dried dextrin (9 to 10 per cent. of moisture) are dissolved in boiling water containing 10 grms. of mixed phosphate buffer (potassium dihydrogen phosphate and disodium hydrogen phosphate) of pH 4.9 to 5.0, and the liquid is cooled and made up to 1 litre. The malt extract is prepared by extracting 20 grms. of the ground malt with 500 c.c. of distilled water for 1 hour in a mechanical shaker. One c.c. of the extract is allowed to act at 40° C. on 70 c.c. of the  $\alpha$ -amylodextrin solution for 1 hour, 3 c.c. of 2 N sodium hydroxide solution being then added to stop the action. The solution is made up to 100 c.c. at 15.6° C., and the cupric oxide reducing power is determined gravimetrically on 20 c.c. of the liquid. The result is calculated as percentage of maltose on the amylodextrin taken. Under these conditions 1 c.c. of malt extract from normal kiln-dried malt usually produces the equivalent of R5 to 10, the action lying well within the limits of the law of proportionality. Extremes in either direction may be corrected by repeating the determination with more or less of the malt extract.

The results obtained with a number of malts indicate that normal kiln-dried malts will always supply sufficient enzyme to liquefy starch paste in the mash tun and so provide soluble starch as substrate for the amylolytic enzymes. The values found for the dextrinolytic activity of certain malts suggest, however, that many mash tun troubles ascribed to shortage of liquefying enzymes might actually be due to a deficiency of dextrinase.

T. H. P.

**Rancidity Changes and the Flavour of Fats.** C. R. Barnicoat. (*J. Soc. Chem. Ind.*, 1931, 50, 361-365r.)—The effect of free fatty acids on the flavour of fats in the absence of oxidation and rancidity was investigated with beef kidney and external fats, mutton kidney fat, and lard. Fatty acids were prepared from these fats with precautions against oxidation. These were mixed with the original fats, and it was found that the flavour of the fats was not impaired with additions up to 10 or 15 per cent. Under similar conditions of temperature and exposure to light the nature of the fat markedly influences the development of rancidity, and the active oxygen and Kreis values, corresponding with the point of first perceptible rancidity, increased from hard beef and mutton kidney fats, through beef external fat, to lard. Readiness to develop a rancid odour and flavour on oxidation appeared greater the smaller the proportion of linolic acid present, and was greatly increased by exposure of the fat to direct sunlight. The substances responsible for the odour and flavour of oxidised fats at ordinary temperatures are regarded as probably products of oxidation of oleic rather than of linolic or linolenic acids. Attempts to determine traces of aldehydes in rancid fats were not satisfactory, partly owing

to the formation of emulsions; a method giving promising results with animal fats but not with methyl oleate, consisted in shaking the fat at concentrations equivalent to about 50 mgrms. of heptaldehyde per litre, in benzene with dilute bisulphite for 12 hours, filtering, and titrating the bisulphite combined with the aldehyde with 0.001 *N* iodine.

D. G. H.

**Susceptibility of Fats to Oxidative Rancidity.** D. P. Grettie and R. C. Newton. (*Oil and Fat Ind.*, 1931, 8, 291-294.)—The incubation test, in which a fat is kept in an oven at a high temperature and inspected for odour and flavour at regular intervals, is long (2 to 40 days), and depends on personal judgment. The method now described is a modification of the methods of Bailey and Issoglio, and consists in passing air over the heated fat and then into dilute permanganate solution, which is titrated with oxalic acid at definite intervals. The air is first purified by passage through a washing bottle containing acid permanganate solution and then passes (1) over the fat dispersed on filter paper in the middle of a tube (20 mm. inside diam.), heated in boiling water, (2) into a test tube (1 inch

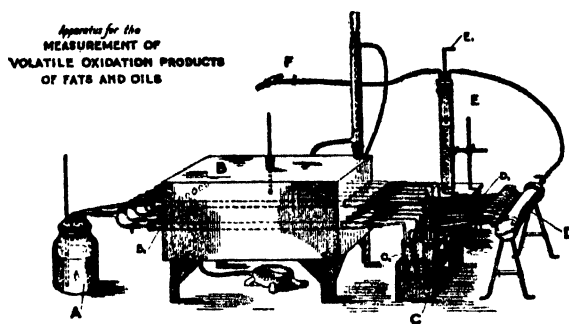


Figure 1

diam.) kept at 25° to 30° C. and containing 10 c.c. of 0.01 *N* potassium permanganate solution acidified with 1 c.c. of approximately 5 *N* sulphuric acid, (3) through a capillary tube, 10 cm. long and of 0.5 mm. bore, (4) through a device for maintaining a constant vacuum, and (5) through a tube, provided with a screw clip and connected with a vacuum pump. A number of samples of fat may be examined simultaneously by using a number of tubes in the boiling water-bath, these tubes being connected at one end with the same acid permanganate air-purifier and at the other end with similar capillary tubes, each with its own acid permanganate condensing arrangement and all passing into a 2-inch iron pipe manifold connected with the vacuum pump. This arrangement ensures the passage of the same volume of air over each sample of fat.

The tube to contain the filter paper and fat is cleaned with acid dichromate and dried. A piece of filter paper, 5 × 30 cm., is creased three times lengthwise to form pleats of four layers and placed across a clean watch glass on a balance, and 2 grms. of the fat are weighed on to it. The fat is distributed so that, when melted, it will be soaked up by the entire paper. This is then carefully introduced into the glass tube, about 8 inches from the end, and the tube placed in the

boiling water oven. After the connections have been made, the stream of air (1 c.c. per second) is started by opening the screw clip on the vacuum tube. At the end of 20 minutes the test-tube containing the acid permanganate is replaced by another. The permanganate solution is then heated in a boiling water bath with 11 c.c. of 0.01 *N* oxalic acid solution until decolorised and at once titrated with standard permanganate. This replacement and titration are repeated at intervals of 20 minutes, until the rate of decomposition of the fat is sufficient to use up 1 c.c. of the 0.01 *N* permanganate in one 20-minute period. The total time of the action before this occurs is taken as an indication of the stability of the fat. When the fat is exceptionally stable, intervals of 60 minutes are used.

After use, the tube in which the fat is heated is left filled with strong acid dichromate cleaning solution overnight, and then rinsed thoroughly with distilled water. In folding the filter paper, several sheets should be creased at once, the outer ones, which the fingers have touched, being discarded. The same type of paper, of purest quality, should be used at all times. The whole surface of the filter paper should be exposed to the air current; if two of the folds adhere at one end or if the flat surface of the paper is pressed against the glass tube, the result is vitiated. When several tubes are heated in the same oven, any one not being used must be closed to the air by a pinchcock between the tube and the air-washing bottle. In case water condenses in one of the capillary tubes and thus prevents the air from passing, this tube must be removed, washed with alcohol, and replaced.

T. H. P.

**Determination of Butyric and Caproic Acids in Edible Fats.** J. Grossfeld and F. Battay. (*Z. Unters. Lebensm.*, 1931, 62, 99-126.)—The difficulty of calculating the butyric acid value of a fat from its Reichert-Meissl value is indicated. It is shown that, for the edible fats, the former value is a summation function of the butyric and caproic acid contents, and that a relation of this type may be used, with the appropriate factors, for the purposes of calculation. The ratios of the partition-coefficients (*K*) of these two acids between water and various organic solvents were then determined, *viz.* ratio for butyric to caproic acid: ether 10, benzene 20, toluene 20, xylene 21, petroleum spirit (b.pt., 40°-60° C.) 26, petroleum spirit (b.pt. 120°-130° C.) 31, light petroleum oil 34. Determinations of *K* were also made for aqueous solutions of the acids of various concentrations, and the law of mixtures was shown to hold for extraction of them from mixed aqueous solutions; the results are tabulated and plotted as curves. On the basis of this work the following method is proposed:—Five grms. of fat are heated in a 300 c.c. Reichert-Meissl flask with 2 c.c. of potassium hydroxide solution (sp. gr. 1.5) and 10 c.c. of volatile acid-free glycerol until saponified, and the soap solution is cooled to 20° C., decomposed and distilled according to Grossfeld's procedure (*id.*, 1927, 53, 382; see also *ANALYST*, 1931, 403). Of the distillate (110 c.c.), (a) 50 c.c. are titrated to phenolphthalein with 0.02 *N* sodium hydroxide solution (*T* c.c.), and 0.616*T* gives the butyric acid value; (b) 50 c.c. are shaken with 50 c.c. of neutral petroleum spirit (b.pt. 40°-60° C.) in a

200 c.c. flask for at least 1 minute, and the aqueous phase again titrated ( $T_1$  c.c.). Then  $A$  (relative decrease in titration) =  $100 (T - T_1)/T$  per cent., allowance being made for blank titrations. If  $K_b$  and  $K_c$  are the decreases in concentration of the water layer when pure aqueous solutions of butyric and caproic acids, respectively, are extracted with petroleum spirit (b. pt.  $40^\circ$ – $60^\circ$  C.) under the above conditions, then the caproic acid ( $C$ ), expressed as a percentage of the total titration ( $T$ ) =  $100(A - K_b)/(K_c - K_b)$ . The percentages of caproic and butyric acids are thence obtained from the formulae  $P_2CT$  and  $P_1(100 - C)T$ , respectively.  $P_2$  is 0.00482 ( $P_1$  0.00129) for butter fat, 0.00548 for coconut oil, 0.00396 ( $P_1$  0.00127) for cocoa butter, and 0.00542 for lard. The data are plotted, and values of  $100(K_c - K_b)$  and  $K_b$  are tabulated. Data provided by the examination of 32 samples of butter fat were examined by statistical methods, and a mean butyric acid content of 3.73 (caproic acid 1.72) per cent. was found; the mean ratio of the percentage of butyric acid to the butyric acid value was 0.186. The method of least squares gave mean deviations of a single value from the mean of all the values of 6.6 for butyric and 16.8 per cent. for caproic acid. Comparison of the contents of the two acids in the butter-fats by the correlation method gave a correlation-factor ( $r$ ) of +0.70, the possible error ( $f$ ) being  $\pm 0.061$ . Caproic acid occurs in edible coconut oil to the extent of 0.61 per cent., and has the lowest molecular weight of any fatty acid present. Examples of the applications of the method (e.g. the detection of fats containing butyric acid in other fats) are given.

J. G.

**The Reaction of Cocaine Hydrochloride Solutions.** H. Rothlin. (*Farmaceutisk Revy*, 1931, 18; *Pharm. J.*, 1931, 127, 190.)—The reaction of aqueous solutions of cocaine hydrochloride to litmus varies from neutral to faintly acid, and the variable statements in the various Pharmacopoeias are regarded as due to the unsatisfactory nature of litmus as indicator. A litmus paper which was not up to the pharmacopoeia standard and was not reddened by 0.001  $N$  hydrochloric acid,  $pH$  3, yet showed a distinct red colour with a 2.5 per cent. cocaine hydrochloride solution of  $pH$  5.5. The 5th edition of the Swiss Pharmacopoeia replaces the litmus test by the determination of the  $pH$  value, and allows a  $pH$  value of 4.6 to 6.4 for a 2.5 per cent. solution of the hydrochloride.

D. G. H.

**Determination of Emetine.** F. C. Sinton. (*J. Assoc. Off. Agr. Chem.*, 1931, 14, 358–360.)—The following method is found to be satisfactory for the determination of emetine hydrochloride in tablets. All the unbroken tablets of the sample are weighed, and the average weight per tablet is calculated. A number of the tablets are powdered and mixed. A weighed amount, sufficient to represent about 0.1 grm. of the emetine salt, is dissolved in a small separating funnel in the minimum quantity of water, 5 c.c. of sodium hydroxide solution (4 grms. of NaOH per 100 c.c.) being then added. The solution is extracted with 30 c.c. of ether (previously washed, by shaking, with an equal bulk of water) and the aqueous solution is drawn off and the funnel swirled to remove water from its sides. The ether is washed with 1 c.c. of water, which is added to the aqueous solution. The



ether is decanted into a second separating funnel, the mouth of the funnel being washed with ether. The extractions are repeated with 25, 20, 15 and 10 c.c. portions of ether or until extraction is complete, washing with 1 c.c. of water each time, and combining the ethereal extracts in the third separating funnel. The total extract, together with ether used to rinse out the funnel, is filtered through cotton wool previously wetted with ether, and evaporated on a steam bath, the evaporation being completed at a low temperature. The residue is treated with 2 c.c. of neutral alcohol, which, with a watch-glass over the beaker, is allowed to reflux on the steam-bath for a few minutes. The liquid is then titrated to a faint pink colour with 0.02 *N* acid in presence of methyl red. The beaker is next covered and the solution is digested on a steam bath until all particles are completely dissolved, and cooled. After addition of 30 c.c. of recently boiled distilled water, the titration is completed to faint redness; 1 c.c. of 0.02 *N* acid  $\equiv$  5.6046 mgrms. of emetine hydrochloride ( $C_{30}H_{44}O_4N_2 \cdot 2HCl$ ). The average of five results obtained by different analysts with a mixture of 1 part of emetine hydrochloride, U.S.P., with 3 parts of milk sugar differed from the theoretical value by not more than 0.2 per cent.

T. H. P.

**Assay of Santonin.** H. M. Burlage. (*J. Assoc. Off. Agr. Chem.*, 1931, 14, 321-325.)—The following procedure is recommended for the assay of santonin in mixtures and tablets of non-fatty nature and, tentatively, in fatty mixtures. A weighed powdered sample, equivalent to approximately 0.15 gm. of santonin, is extracted with 10, 10, 10, 5, and 5 c.c. portions of petroleum spirit saturated with santonin (if the sample is fat-free this step may be omitted). Each portion of solvent is filtered, with the aid of suction, to complete dryness through a Gooch crucible provided with an asbestos mat, before following with another portion. The residue in the extraction flask and crucible is extracted with 15, 10, 5, and 5 c.c. of hot benzene, each portion being filtered as before. The benzene extract is transferred to a tared flask and, after evaporation of the solvent, the residue of santonin is dried to constant weight at 100° C. As a check, the residue is dissolved in 25 c.c. of warm aldehyde-free, neutral alcohol, the solution being neutralised and treated with 5 drops of phenolphthalein and 25 c.c. of 0.1 *N* potassium hydroxide solution. The liquid is digested on a water bath under a reflux condenser for 30 minutes, and then titrated, while hot, with 0.1 *N* hydrochloric acid, in presence of 5 drops of phenolphthalein solution. A blank test with 25 c.c. of aldehyde-free neutral alcohol and 25 c.c. of the alkali solution is made similarly.

T. H. P.

**Assay of Tablets of Resin of Podophyllum.** L. E. Warren. (*J. Assoc. Off. Agr. Chem.*, 1931, 14, 380-386.)—Tablets and pills of this resin usually contain starch, starch paste, talc, acacia, and liquid paraffin as binders, fillers, excipients, etc., and other laxatives, such as aloe, extract of colocynth, cascara or leptandra, resin of jalap or ipomoea, etc., are often present. The following procedure for extracting the resin and for determining the resin in the extract yields satisfactory results when other laxatives are absent:—A weighed quantity of the

powdered tablet material, representing about 0.75 gm. of resin of podophyllum, is mixed in a beaker with 10 grms. of fine washed sand, and the whole is transferred to a 30 c.c. Gooch crucible. Alcohol (25 c.c. in all) is poured in small portions through the crucible and is collected in a 100 c.c. measuring flask. After draining, the crucible is transferred to a Bailey or Soxhlet extractor, and extraction with 60 c.c. of alcohol is continued until the residue is completely exhausted of resin. This alcoholic solution is also placed in the 100 c.c. measuring flask and the whole is made up to volume with alcohol.

Ten c.c. of the tincture thus prepared are shaken in a separating funnel with 10 c.c. of chloroform and 10 c.c. of 0.6 per cent. hydrochloric acid (2 c.c. of the strong acid and 100 c.c. of water) and allowed to separate. The lower layer is transferred to another separating funnel, and the upper layer is extracted in the first funnel with three 15 c.c. portions of a mixture of 1 volume of alcohol and 2 volumes of chloroform, these extracts being added to the liquid in the second funnel. This liquid is now shaken with 10 c.c. of 0.6 per cent. hydrochloric acid and, after separation, the lower layer is drawn off into a weighed beaker or Erlenmeyer flask and the acid liquid is extracted with three 15 c.c. portions of fresh alcohol-chloroform mixture. The combined chloroform extractions are evaporated, the container being rotated in an inclined position as the last portions are dissipated. The residue is treated with 1 c.c. of dehydrated alcohol, which is then evaporated, the residue being finally dried to constant weight at 80° C.

By this procedure, nine brands of tablets of resin of podophyllum were found to contain from 83 to 124 per cent. of the resin contents claimed, the limits for seven of these being 90 and 112 per cent.

T. H. P.

**Amounts of Nicotine in Tobacco Smoke. G. Pyriki.** (*Z. Unters. Lebensm.*, 1931, 62, 95-98.)—Four oriental tobaccos, containing 0.06 to 1.28 per cent. of nicotine, were blended to give mixtures containing 0.06 to 1.08 per cent. of nicotine (moisture contents 11.3 to 12.2 per cent.), and from these cigarettes were made, 6.5 cm. long. These were "smoked" artificially in a stream of air (5 at a time, with mean total weight of 5.7 to 6.4 grms.) by the method of Pfyl and Schmitt (*ANALYST*, 1927, 52, 728). The experiment was stopped when 5.2 cm. had been consumed, and pauses of 6 seconds were made every 4 seconds. For quantities up to 0.25 and 0.5 per cent. of nicotine in the original tobacco, 5 mgrms. per 100 grms. of dry tobacco were always found in the smoke obtained when the experiment was carried out in 5 and 8 minutes, respectively; a blank experiment on "nicotine-free" tobacco gave the same value. The corresponding (mean) values for tobaccos containing 0.3, 0.47, 0.6, and 1.1 per cent. of nicotine were 6, 25, 55, and 235 (for 5 minutes), and 5, 5, 40, and 174 (for 8 minutes), respectively. It is, therefore, considered that the proposed maximum limits for cigarette tobaccos described as "nicotine-free" (0.08 per cent.) and "poor in nicotine" (0.20 per cent.) are too low, and that 0.10 to 0.15 and 0.5 to 0.6 per cent., respectively, would be more suitable.

J. G.

## Biochemical.

**Experimental Observations on Dermatitis due to Dyed Fur.** G. H. Percival. (*Lancet*, 1931, 221, 417-424.)—The experiments described were made (1) with dyed and undyed furs, and (2) by means of Jadassohn and Bloch's contact eczema procedure, in which the suspected irritant in low concentration is applied as solution, paste or ointment to a small area of the skin of the back. The application is covered with linen or oiled silk held in position with adhesive plaster, and is removed after 24 hours for examination of the skin. A positive test is indicated by a red and slightly oedematous condition of the skin, the surface of which is studded with minute vesicles. The individuals on whom the experiments were made were either normal, or subject to fur dermatitis itself or to skin disease of another type.

The results obtained lead to the following conclusions:—The potential irritant properties of a dyed fur are due to some substance used in the dyeing process and not to mechanical irritation by the fur. These irritant properties are enhanced by moistening the fur with water or saline solution, but such preliminary moistening is not essential for the production of a positive reaction. Normal skins do not, however, react to a dyed fur under conditions which would cause dermatitis in a fur dermatitis patient. Dermatitis is hence due to a hypersensitive skin, the reaction being allergic in nature. With furs dyed with a particular paraphenylenediamine compound (A) of the ursol type, it is either this compound itself or an early oxidation product of it which reacts with the allergic skin. Fur dermatitis cases react to concentrations of A varying from 0.005 to 0.5 per cent., there being considerable individual variation in the concentration necessary to produce dermatitis. Most normal skins fail to react with a 10 per cent. solution of A, although some react with a 5 per cent. solution. The degree of sensitivity of the skin of dermatitis cases to dyed fur ranges from at least 200 times to 2000 times that observed with non-fur dermatitis cases. It is possible for at least a 0.5 per cent. solution of A or its oxidation products to be formed in the fluid secretions of the skin when in contact with the dyed fur; such solution is without effect on non-fur dermatitis cases. The dermatitis resulting from a single application of a minute concentration of a chemical towards which the skin is allergic may persist for several weeks. The skin of a patient suffering from dermatitis caused otherwise than by fur is not specially apt to react to dyed fur, a specific capacity, bearing no relationship to the oiliness or humidity of the skin, being a necessary factor for reaction to occur. That positive reactions were obtained only when A was applied to the intact skin and that uniformly negative results were found by the scratch method, points to the allergy being localised in the epidermis.

By means of a cleansing process consisting in washing the dyed fur in water or benzene, and then brushing or drumming with sand, the harmful substance may be greatly reduced in amount, and, possibly, completely eliminated.

T. H. P.

**Rôle of Copper in Haemoglobin Regeneration and in Reproduction.**

**H. L. Keil and V. E. Nelson.** (*J. Biol. Chem.*, 1931, 93, 49-57.)—The relation of copper to haemoglobin formation has attracted the attention of a number of investigators during the past few years, and only a superficial examination of the literature is necessary to reveal that there is considerable divergence of opinion regarding the rôle and necessity of copper in haemoglobin building. Experiments by the authors show the following results:—Nutritional anaemia is readily produced in rats on a whole milk diet. Pure iron as ferric chloride, when added to milk collected in glass, does not cause regeneration of haemoglobin. Salts of vanadium, titanium, manganese, nickel, arsenic, germanium, zinc, chromium, cobalt, tin and mercury also failed to stimulate regeneration of haemoglobin when added to milk collected in glass, and supplemented with pure iron as ferric chloride. Copper was the only element of those tested which had a positive effect on haemoglobin building. Reproduction was obtained on milk and iron as ferric chloride by the sole addition of copper sulphate (0.05 mgrm. daily). This was in contrast to the results of Krauss (*J. Dairy Sci.*, 1929, 12, 74, 242), who did not obtain reproduction when rats were fed on a milk diet supplemented with copper and iron. Nutritional anaemia produces a distinct change in colour of the dark coated rats. Black or black-hooded animals replace the black colour with a silvery grey that is very striking. Grey-coated animals change to a buff or silvery grey with a yellowish tint. Animals on whole milk, plus pure iron as ferric chloride, also develop this change in the colour of the fur. The addition of 0.05 mgrm. of copper per day to the whole milk and iron as ferric chloride restores the original coat in about 2 months' time.

P. H. P.

**Colorimetric Determination of Calcium in Blood. S. Yoshimatsu.**

(*Tohoku J. Exp. Med.*, 1930, 15, 355; *Mikrochem.*, 1931, 9, 529.)—Calcium is determined in 0.1 c.c. of blood, diluted with 0.7 c.c. of water. The proteins are precipitated by adding 0.1 c.c. of 10 per cent. sodium tungstate and 0.1 c.c. of  $2/3$  *N* sulphuric acid, and, after 15 minutes, the mixture is filtered through a small glass filter, and the precipitate is washed three times with 0.3 c.c. of water. To the combined filtrates 0.3 c.c. of a 33 per cent. solution of Rochelle salt, 0.5 c.c. of 1 *N* sodium hydroxide solution, and 0.3 c.c. of a 5 per cent. alcoholic solution of oxyquinoline are added, drop by drop. The calcium oxyquinoline compound produced is separated by centrifuging and washed four times with an alkaline solution of Rochelle salt, 1 c.c. being used each time. The precipitate is then mixed with 1 c.c. of ammoniacal ammonium chloride solution (1 litre of 5 per cent. ammonium chloride mixed with 34 c.c. of ammonia solution of sp. gr. 0.9), and boiled for 5 minutes, one drop of ammonia solution being added twice during the heating. The mixture is then filtered through a glass filter, washed twice with 0.5 c.c. of water and then dissolved in 0.5 c.c. of 0.01 *N* hydrochloric acid, and the solution is treated with 1.2 c.c. of a 20 per cent. solution of sodium carbonate and 1 c.c. of Folin-Denis' phenol reagent (*J. Biol. Chem.*, 1915, 22, 305). The mixture is then warmed for five minutes, and, after cooling, the colour is compared with that of a standard.

J. W. B.

**Microchemical Determination of Magnesium in Blood without removing Calcium.** S. Yoshimatsu. (*Tohoku J. Exper. Med.*, 1929, 14, 29; *Mikrochem.*, 1931, 9, 528.)—For each determination 1 c.c. of blood is diluted with 7 c.c. of water, and 1 c.c. of 1 per cent. sodium tungstate solution and 1 c.c. of  $2/3 N$  sulphuric acid are added. The mixture is centrifuged, and 0.3 to 0.5 gm. of ammonium chloride is added to 5 c.c. of the supernatant liquid. The solution is then boiled, and 7 drops of ammonia solution (sp. gr. 0.96) and 7 drops of an alcoholic solution of oxyquinoline are added. The mixture is left on the water bath for 10 minutes, and after each 3 minutes one drop of ammonia solution is added. The precipitate is then centrifuged, while hot, washed 3 times with 2 c.c. of a 5 per cent. ammonium acetate solution, and then dissolved in  $N$  hydrochloric acid. The solution is mixed with 5 c.c. of a 25 per cent. sodium carbonate solution and 1 c.c. of Folin-Denis phenol reagent (*J. Biol. Chem.*, 1915, 22, 305), and warmed for 30 seconds. The colour is compared, after cooling, with that given by a standard. J. W. B.

**Effect of Ultra-Violet Irradiation upon the Free Sterols of Lanolin.** A. Bernhard and I. J. Dreker. (*J. Biol. Chem.*, 1931, 93, 1-3.)—It has previously been shown that following lanolin injections and ultra-violet irradiation there is a rise in the blood sterol (cholesterol) of children and adults. The nature of this rise in sterol content after irradiation has been investigated further. Lanolin consists of different fatty acids, cholesterol, cholesterol esters, oxysterol, so-called ischolesterol, agnosterol and lanosterol. (The last two are not precipitated by digitonin.) Lanolin also contains other alcohols, such as lanolin alcohol, carnaubyl, ceryl, and undetermined higher alcohols, all of which make up about 50 per cent. of lanolin. The total sterol content found, after saponification, is about 20 per cent. The results show that the amount of free sterols in anhydrous lanolin which is precipitated by digitonin, expressed in terms of free cholesterol, is 0.93 per cent. of lanolin. When anhydrous lanolin was irradiated with ultra-violet light there was a progressive gradual rise in the amount of free sterols precipitated by digitonin; the maximum rise was reached at the end of 1 hour. The free sterols, expressed as free cholesterol, increased from 0.93 to 5.37 per cent. of lanolin. Three weeks after irradiation the amount of sterols precipitated by digitonin had not changed. Colour reactions for ergosterol in the lanolin, both before and after irradiation, were not specific, a brownish-green colour of the same intensity being obtained in both instances. The crystalline fractions obtained in attempts to isolate cholesterol from lanolin which had been irradiated for 1 hour could not be identified as pure cholesterol. Therefore, digitonin precipitates substances other than cholesterol. P. H. P.

**Vitamin A and the Antimony Chloride Reaction.** A. Emmerle, M. v. Eekelen and L. K. Wolff. (*Nature*, 1931, 128, 495-496.)—The blue solution obtained with vitamin A and antimony trichloride shows two absorption bands at  $572\mu\mu$  and  $610\mu\mu$ , respectively, and the blue colour depends on the intensity of the  $610\mu\mu$  band. Recently, however, Morton and Heilbron have stated that the strength of different vitamin A preparations corresponds better with the extinction

of the mixture in the  $572\mu\mu$  region than in the  $610\mu\mu$  region, and the following facts confirm this:—By treatment of a vitamin *A* preparation from saponified cod-liver oil or saponified extract of cow's liver with some drops of furan, methylfuran, pyrrole, indole, or skatole (all substances of related chemical structure), and the addition of antimony chloride, the mixture turns purple instead of blue, and in the spectroscope the  $610\mu\mu$  band is no longer seen, whereas the  $572\mu\mu$  band remains unaltered. The physiological activity is unimpaired and the preparation (without antimony chloride) shows the  $328\mu\mu$  band just as the original substance did. It is known that liver may contain indole-like substances, and so it can be understood that in some vitamin *A* preparations the  $572\mu\mu$  band is the stronger, and in others the  $610\mu\mu$  band is the stronger.

P. H. P.

**Determination of Vitamin A in Butter.** B. G. E. Morgan and K. H. Coward. (*Lancet*, 1931, 221, 758–759).—The method described by Coward, Key, Dyer and Morgan (*Biochem. J.*, 1930, 24, 1952; 1931, 25, 551), and Coward, Dyer, Morton, and Gaddum (*Biochem. J.*, 1931, 25, 1102) for the determination of vitamin *A* in cod-liver oils has been shown to give consistent results also in the determination of vitamin *A* in butters and margarines. This is of value, for the testing of different amounts of butter involves the giving of different amounts of fat, in addition to the fat-free diet employed in this method, whereas the testing of different doses of cod-liver oils has always been carried out by diluting, so that the required dose is contained in the same amount (19 to 21 mgrms.) of olive oil. The manufacturers of the "Gold Chain" margarine used for the test claim for it a vitamin *A* potency equal to that of butter. The figures in a table giving the measurement of the vitamin *A* content of different doses of butter and margarine by reference to a standard sample of cod-liver oil, show that their claim is justified. A dose of 0.05 grm. of butter proved to be equivalent to a dose of 0.05 grm. of margarine (measured as being equal to 1.4 and 1.5 mgrms. respectively of the standard sample of cod-liver oil), and a dose of 0.1 grm. of butter proved to be equivalent to a dose of 0.1 grm. of margarine (measured as 3.5 and 3.4 mgrms. respectively of the standard sample of cod-liver oil). Incidentally it is of interest also to note that the vitamin *A* potency of the sample of butter, and also of the sample of margarine, is about one-thirtieth ( $1.4/50$ ,  $3.5/100$ ,  $1.5/50$ ,  $3.4/100$ ) of the sample of cod-liver oil which was adopted by the authors as being an average medicinal cod-liver oil.

P. H. P.

**Application of the Uranyl Zinc Acetate Method of Determining Sodium in Biological Material.** See p. 764.

## Toxicological.

**Determination of Carbon Monoxide produced from Painted Surfaces in Confined Spaces.** F. H. Newington. (*J. Soc. Chem. Ind.*, 1931, 50, 371–375r.)—A fatality which occurred when a workman entered a closed compartment of one of H.M. ships was found to be due to carbon monoxide poisoning. The compartment had been painted with paint composed of oxide of iron pigment, boiled linseed oil, and paste driers, and was closed while the paint was wet and kept

closed during the ensuing five years. Investigation of the air in a number of such compartments and of the air in an iron boiled linseed oil drum which had been emptied and left closed for a few weeks, has shown that the carbon monoxide originates from the linseed oil, and that this gas may be formed from either the boiled or the raw oil during the process of drying. The compartment in which the fatality occurred was afterwards repainted and closed (without vent) for six months, the air then giving the following results on analysis: Nitrogen, argon, etc., 86.47; oxygen, 8.72; carbon dioxide, 4.56; carbon monoxide, 0.131; hydrogen, 0.07; saturated and unsaturated hydrocarbons, below 0.05 per cent. The air from a compartment on another ship which had been closed (with small vent) for 2 years contained: Nitrogen, argon, etc., 98.37; oxygen, 1.28; and carbon monoxide, 0.033 per cent. The amount of carbon monoxide produced from linseed oil during drying in a closed space is controlled by the quantity of oxygen available for absorption by the oil. Provided excess of the oil is present, as must be the case in a compartment coated internally with paint, the maximum concentration of the monoxide to be expected appears to be about 0.3 to 0.4 per cent.

As the amount of carbon monoxide to be expected in these atmospheres was very small, the method of determining it was based on the delicate reaction with iodine pentoxide, the iodine formed being absorbed in potassium iodide solution and subsequently titrated with *N*/500 thiosulphate solution in presence of starch. Owing to the large number of other gaseous constituents possibly present in the compartments, special precautions were necessary to ensure the removal by the purifying train of any gas or vapour other than carbon monoxide that might react with iodine pentoxide. Contact of the gases with rubber joints or other organic matter must be avoided. The apparatus, specially designed to be portable and to be usable on board ships in full commission, is described in detail; in essentials the method used is similar to that described by Davies and Hartley (*J. Soc. Chem. Ind.*, 1926, 45, 164r).

T. H. P.

**Toxicological Detection and Isolation of Barbital.** J. J. L. Zwikker. (*Pharm. Weekblad*, 1931, 68, 975-983.)—*Detection*.—A pale red colour is given by barbital and its phenyl and allyl derivatives on addition of cobalt chloride in methyl alcohol solution free from water. If a filtered saturated solution of barium methylete, prepared by shaking barium oxide with water-free methyl alcohol, is then added, a stable, deep indigo-blue colour results. Urine (10 c.c.) should first be cleared with lead acetate, then extracted with 10 c.c. of ether, and the residue after evaporation dissolved in 1 c.c. of the methyl alcohol and tested. The test is sensitive to 0.5 mgrm. of barbital, and could probably be adapted to a colorimetric determination, though no details are given. A blank test should give only a dirty yellow colour. *Isolation*.—The reagent is 4 c.c. of 10 per cent. copper sulphate solution with 1 c.c. of pyridine and 5 c.c. of water, and 2 to 4 drops are added to 1 mgrm. or more of veronal or dial in 1 c.c. of water, when a sparingly-soluble, purple-red precipitate of  $(C_8H_{12}O_3)_2 Cu(C_5H_5N)_2$  results. This is washed with acid to liberate the barbital, which is removed, dried at 105° C., and weighed.

The m.pt. may then be found and the tests described by Van Itallie and Steenhauer (ANALYST, 1930, 55, 717) applied. Urine (50 c.c.) is first cleared with 10 per cent. by volume of lead acetate solution, the filtrate shaken twice with 25 c.c. of ether, the extract is evaporated, and the residue is dissolved in water containing pyridine and precipitated as described. The reaction is specific and quantitative for 1 mgrm. of drug, and is given in the presence of caffeine, theobromine, santonin, and of tartaric and succinic acids.

J. G.

#### **Toxicity of Methyl Alcohol following Skin Absorption and Inhalation.**

C. P. McCord. (*Ind. Eng. Chem.*, 1931, 23, 931.)—Thirty-one monkeys, 58 rabbits and 176 white rats were subjected to the action of various preparations of methyl alcohol, commercial and synthetic, by inhalation and by skin absorption. It was found that pure methyl alcohol is definitely toxic to animals most like men, and it is assumed that practical hazards for human beings may be produced under conditions of trivial exposure. Methyl alcohol (but no formaldehyde) was regularly recovered on distillation of all the organs tested from skin-treated animals, none being found in the controls. A quantity of 0.5 c.c. per kilo. of body weight, applied four times daily, will produce illness in monkeys followed by death. The threshold of danger by inhalation is well below 1000 parts of methyl alcohol vapour per million.

R. F. I.

## **Bacteriological.**

**Chemical Changes in the Fat of Frozen and Chilled Meat. Part III, Frozen Bacon.** C. H. Lea. (*J. Soc. Chem. Ind.*, 1931, 50, 343-349T; cf. ANALYST, 1931, 56, 538, 610).—Tank-cured bacon stored at  $-10^{\circ}\text{C}$ . for periods up to 152 days, followed by hanging at  $15^{\circ}\text{C}$ . for 18 days, showed no evidence of attack of micro-organisms on the superficial layers of fat, and the rise in free acidity of the fat was of the same order as that previously observed for lamb fat. Oxidation has usually set in on the exposed fat at the end of curing, and at  $15^{\circ}\text{C}$ . it proceeds rapidly in the absence of light, in sharp distinction to the case of the fat of fresh beef and mutton. The fat at the same time turns yellow on the surface, and sometimes yellowing affects the interior of the fat. The oxidised fat has an unpleasant rancid flavour on cooking. The effect of smoking after storage at  $-10^{\circ}\text{C}$ . was to arrest superficial oxidation for a time, and there is some evidence that interior oxidation may be retarded to some extent, presumably owing to the presence in the smoke of small quantities of anti-oxidants, possibly aromatic phenols. Rapid cooling of the carcasses at  $5^{\circ}\text{C}$ . prior to curing appeared to have no effect on the fat. Addition of small quantities of cod-liver oil to the diet of the pigs did not affect the iodine value of the fat of the bacon, but the fat had a tendency to oxidise more readily at the inner centre, to develop a slightly deeper yellow colour on the exposed edge of the back fat, or gave an oily flavour on cooking. Probably such defects would not appear if the cod-liver oil were excluded from the diet some weeks prior to slaughter.

D. G. H.



**Yeasts found in Fermenting Honey.** G. E. Marvin, W. H. Peterson, E. B. Fred, and H. F. Wilson. (*J. Agric. Res.*, 1931, 43, 121-131.)—Crystallisation of honey and fermentation were found to be related, for, on granulation, the water formerly serving as solvent for the glucose crystals is retained in the liquid part, and increases the water content of the syrup until the syrup becomes dilute enough to permit of the growth of yeasts which could not grow in the un-crystallised honey. The fermentation process is slow (from six months to several years), and the chief fermentation products are about equal quantities of carbon dioxide and alcohol (not often over 5 per cent.) and small quantities of non-volatile acids. The morphological characters of the 5 yeasts isolated were studied, and what appear to be two new species are described. Fermentation may be prevented by keeping the honey at 100° F. for several months, or at 122° F. for 24 hours, or by heating to 160° F. In the latter case, if heating is rapid and the honey is poured into the containers and sealed while hot, and cooled soon, the colour and flavour will not be greatly changed. Unheated honey should be stored at a temperature below 52° F., as honey yeasts will not grow below this temperature. D. G. H.

## Organic Analysis.

**New Colour Reaction for Soluble Organic Sulphur Compounds.** I. W. Grote. (*J. Biol. Chem.*, 1931, 93, 25-30.)—A new colour reaction has been found, apparently limited to compounds of divalent sulphur doubly linked to a single non-metallic element. The active reagent is prepared as follows: 0.5 gm. of sodium nitroprusside (sodium nitroferrocyanide) is dissolved in 10 c.c. of water at room temperature, 0.5 gm. of hydroxylamine hydrochloride is added, followed by 1 gm. of sodium bicarbonate. After evolution of gas has ceased, 2 drops of bromine are added. Excess bromine is removed by aeration and the dark greenish or black-brown solution filtered and made up to 25 c.c. This solution behaves like that prepared by exposure of sodium nitroprusside to sunlight, and contains a mixture of several compounds, one of which reacts with the thiourea type and another like ordinary nitroprusside. The solution is stable for about 2 weeks. With the use of the new reagent a method is described to distinguish soluble organic compounds of the types C-S-H, C-S-S-C, and C=S from other types and from one another. The following is the method:—5 to 20 mgrms. of the compound to be tested are dissolved in 2 to 3 c.c. of water, and solid sodium bicarbonate is added to excess. About 0.5 c.c. of the general test reagent is then added. A purple-red coloration, produced instantly or within 10 minutes, indicates C-S-H, whilst an intense green or blue indicates C=S or E=S (where E is any single non-metallic element). Both colours may fade more or less rapidly, but often re-appear upon the addition of fresh reagent. If no colour appears within 10 minutes, an equal volume of 5 per cent. potassium cyanide solution is added. C-S-S-C compounds will give a pink to purple-red coloration within 30 minutes. Ring-linked sulphur compounds, both of the C-S-H and the C-S-S-C type, may fail to react, such as diparatolyl disulphide. Organo-metallic compounds, such as the type -C-S-Bi=R<sub>3</sub>,

may require a preliminary warming with dilute ammonia before reacting. The new reagent may be used for quantitative colorimetric determination of thiosulphate, thiocyanate, thiourea, and other compounds of the  $C=S$  type. The colour with the  $C=S$  type is usually first green, then turquoise, and finally deep blue; it may stop at the deep green or turquoise stage, and, in a few cases, as with thiourea, it may go through the blue stage to a purple-red and finally crimson after several hours. Some compounds destroy the reagent, but many of these give transient colours of the correct type. No colour is given with sulphones, sulphonic acids, urea, barbituric acid, taurine, thiophene, or *iso*-thioureas. Among the compounds tested and found in the proper class are: (1)  $C-S-H$ : cysteine, glutathione, thio-glycollic acid, thiosalicylic acid, butyl mercaptan, and bismuth sodium thio-glycollate. Thiophenol and thiocresol give fugitive purple-red colours only, the reagent being rapidly destroyed. (2)  $C-S-S-C$ : cystine, diglycylcystine, dialanyl-cystine, dithioglycolic acid, dibutyl disulphide, dibenzyl disulphide, diformidine disulphide. (3)  $C=S$  and  $E=S$ : thiourea, allyl thiourea, tetramethyl thiourea, thiocarbanilide, thiobarbituric acid, thioacetic acid, potassium xanthate, sodium azido carbon disulphide, thioacetamide, potassium thiocyanate and sodium thio-sulphate. Phenyl-isocyanate destroys the reagent in alcoholic solution giving a transitory blue coloration. Sodium diethyldithiocarbamate destroys the reagent, without coloration.

P. H. P.

**Examination of Commercial Egg-Yolk.** R. F. Innes. (*J. Inter. Soc. Leather Trades Chem.*, 1931, 15, 434.)—In addition to those constituents of egg-yolk which are usually determined (moisture, fatty matter, mineral matter, and sodium chloride) the author suggests that determinations of nitrogen and phosphorus should be made, that the constants of the fatty matter be determined (unsaponifiable matter, free fatty acids, total fatty acids, including their m.pt. and iodine value) and that there should also be an emulsification test. The following figures were found for the yolk of fresh eggs expressed as percentages:—Moisture, 50.50; fatty matter, 26.20; mineral matter, 2.52; chlorides (as sodium chloride), 0.20; nitrogen, 2.61; and phosphorus, 0.58. The fatty matter contained 85.3 per cent. of total fatty acids, 0.90 per cent. of free fatty acids, and 6.43 per cent. of unsaponifiable matter. The total fatty acids had a m.pt. of  $38^{\circ}C$ . and iodine value (Wijs) 63.40. On adding one drop of the sample to 5 c.c. of water in a watch glass containing a trace of sodium chloride a spontaneous emulsion formed.

Four commercial egg-yolks were submitted to this scheme of examination. In two cases, which gave satisfactory results in dressing leather, the figures given were similar to the above. The remaining two gave greasy and unsatisfactory leathers, there was a deficiency of nitrogen, phosphorus and unsaponifiable matter, and the iodine value of the total fatty acids was much higher than with the others. No spontaneous emulsion was formed with water.

R. F. I.

**The Furfural Number of Tanning Extracts and their Mixtures with Sulphite Cellulose Extract.** V. Némec. (*J. Inter. Soc. Leather Trades Chem.*, 1931, 15, 440.)—The method of Laufmann, in which use is made of the

formation of condensation products of tannins with furfural is modified. Fifty c.c. of the unfiltered analytical tannin solution are placed in a conical flask together with 40 c.c. of a freshly prepared mixture of 7 per cent. aqueous furfural solution and 20 per cent. hydrochloric acid (equal volumes). The mixture is boiled over a naked flame, cooled and filtered through S and S 588 folded filter paper of 12.5 cm. diameter, 30 c.c. of the filtrate are evaporated, and the residue is dried and weighed "C". The total solids in 25 c.c. of the untreated tannin solution are designated "A", and the residue from 25 c.c. of the tannin solution and 20 c.c. of the furfural reagent is designated "B". The furfural number "F" equals

$$\frac{100 \times (B - 1.5 C.)}{A}$$

In the case of quebracho, mimosa bark, and gambier, the value of F lies between 94 and 105. For chestnut, sumac, valonia and myrobalans it lies between 2 and 9. Other values are:—Mangrove bark 62, pine bark 41, sulphited quebracho 93 to 96, sulphite cellulose —11 to —16. The test thus forms a means of quantitatively determining sulphite cellulose in pure tanning extracts. R. F. I.

**Decay of Book-binding Leathers.** R. W. Frey and I. D. Clarke. (*J. Amer. Leather Chem. Assoc.*, 1931, **26**, 461.)—A series of ten dummy books covered with various leathers was exposed in a rack near a west window, open during the day, for 8½ years. At the end of this period all the bindings had become more or less deteriorated in the exposed (back) portion. An analysis was made of the backs, the protected sides, and the original leather preserved in sealed envelopes, for Procter-Searle acidity, total sulphur, total and fixed sulphates in the water extract, mineral matter, and fats and a determination was made of the class of tanning material used. The results of certain physical tests are also recorded—crackiness, "scuffiness," tensile strength, resistance to tear, porosity, and permeability. Nos. 1, 2 and 3 had suffered least, Nos. 5 to 10 the most, and No. 4 intermediate. The analyses may be summarised as follows:

Acidity (Procter-Searle), per cent.	1	2	3	4	5	6	7	8	9	10
as sulphuric acid. Sheep.	Goat.	Goat.	Goat.	Pig.	Sheep.	Goat.	Goat.	Cow.	Cow.	
Back .. ..	2.57	2.01	2.54	4.09	6.13	8.62	7.73	7.27	5.26	9.38
Sides .. ..	0.36	0.00	0.51	0.88	2.14	2.31	2.49	1.16	1.78	2.32
Original ..	0.31	0.19	0.26	0.34	1.39	1.31	1.38	0.00	1.27	1.55
Original leathers—										
Ash .. ..	5.43	6.52	0.42	0.41	0.25	1.92	0.60	0.50	1.76	1.24
Fat .. ..	9.16	9.46	0.96	12.29	3.14	11.96	11.96	7.13	8.38	14.70
Tannage										
Pyrogallol			Mixed Pyrogallol			Catechols				

It is pointed out that decay runs parallel with sulphuric acid absorption from the outdoor atmosphere, and that the magnitude of this absorption in the comparatively short period of 8½ years was quite remarkable. The Procter-Searle

values of the backs of Nos. 1 and 2 are much lower than the others. It is considered that this may be due to their high content of ash containing sodium chloride and calcium, since the fixed sulphates in the aqueous extracts of these are high. (The fixed sulphates are those left after evaporating and igniting the water extract and dissolving the residue in water.) Apart from these two, the only explanation for the variation in sulphuric acid absorption of Nos. 3 to 10 would appear to be the nature of the tanning material. Resistance to decay appears to be independent of the fat content, since leathers with both high and low fat content are found to have both high and low resistance. The use of a pyrogallol type of tanning material does not ensure resistance to rotting, since No. 5 rotted badly.

Determinations of nitrogen were made on the leathers before and after extraction with 0.1 *N* sodium carbonate solution. In a bad leather (No. 10) the percentage of total nitrogen in the original leather was 8.39, in the sides after extraction 8.33, and in the back after extraction only 2.19. Such loss becomes appreciable at 65 per cent. deterioration, as found by loss of tensile strength.

R. F. I.

## Inorganic Analysis.

**New Method for the Detection of Cobalt. T. Bersin.** (*Z. anal. Chem.* 1931, 85, 429-433.)—Cobalt may be detected in a solution, from which metals of Group II (the hydrogen sulphide group) are absent, by rendering it ammoniacal after the addition of ammonium chloride (the presence of precipitated hydroxide of iron, aluminium, etc., may be disregarded), boiling for about half-a-minute to oxidise the cobalt, and adding a solution of the anilide of thioglycollic acid in alcohol. The presence of cobalt is shown by the formation of a reddish-brown flocculent precipitate which does not dissolve when the liquid is subsequently acidified. The sensitiveness of the test may be enhanced by shaking the acidified solution with ether, chloroform, or benzene, when the precipitate collects at the junction of the solution and the organic liquid. The method is capable of detecting 0.5γ of cobalt in 5 c.c. of solution containing up to 0.1 grm. of nickel; in the presence of about 10 mgrms. of aluminium, zinc, chromium, ferrous iron, manganese and nickel in 5 c.c., the detection of less than 1 mgrm. of cobalt is uncertain. A simple method of preparing the reagent is described.

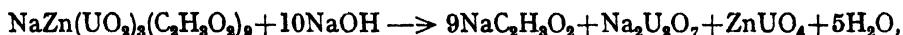
S. G. C.

**Quantitative Separation of Lead and Iron. H. Funk and O. v. Zur-Mühlen.** (*Z. anal. Chem.*, 1931, 85, 435-438.)—The separation depends on precipitating the lead as chromate, employing a somewhat higher concentration of acetic acid in the solution than is usual, in order to prevent the co-precipitation of iron as basic ferric acetate. To the slightly acid solution containing the lead and iron as chloride or nitrate, ammonium acetate is added, followed by 10 c.c. of glacial acetic acid; the liquid is diluted to 100 c.c. and heated almost to the boiling point. The lead is precipitated by slowly adding an excess of dilute ammonium dichromate solution (1 per cent.), with stirring. After cooling, the lead chromate is filtered off and washed with cold water containing a little acetic acid. The lead

can be determined either by weighing the precipitate or by the iodimetric titration of the chromate radicle in it; the iron in the solution can be precipitated as hydroxide and weighed as oxide or determined volumetrically. Good results were obtained with 0.05 grm. to 0.2 grm. of lead mixed with 0.14 grm. to 0.05 grm. of iron.

S. G. C.

**Volumetric Method of Determining Sodium.** J. T. Dobbins and R. M. Byrd. (*J. Amer. Chem. Soc.*, 1931, 53, 3288–3291.)—The method is based on the precipitation of sodium as sodium zinc uranyl acetate in the usual way, and the titration of this with sodium hydroxide. The precipitate is washed three or four times with 2 c.c. portions of ethyl alcohol (95 per cent. saturated with sodium zinc uranyl acetate) and dissolved in water (about 100 c.c.) After the addition of five drops of phenolphthalein indicator, the liquid is titrated with 0.5 *N* sodium hydroxide solution until a red colour is produced which persists after heating the liquid to incipient boiling for five minutes; the excess of alkali which is then present is titrated with standardised dilute hydrochloric acid, the end-point being ascertained by allowing the yellow precipitate in the solution to settle after each addition of the acid, and viewing the supernatant liquid. The amount of sodium present can be calculated from the equation



it having been found that 10 grm.-mol. of sodium hydroxide are required for each grm.-mol. of the triple salt. It is recommended, however, to standardise the alkali solutions used by means of the solution of the precipitate of sodium zinc uranyl acetate given by a known amount (20 to 25 mgrm.) of sodium chloride, in order to allow for the personal error in determining the end-point of the titration. The method is claimed to be superior to that in which the sodium zinc uranyl acetate is weighed, since it is independent of the somewhat uncertain degree of hydration of the triple salt.

S. G. C.

**Application of the Uranyl Zinc Acetate Method of Determining Sodium in Biological Material.** A. M. Butler and E. Tuthill. (*J. Biol. Chem.*, 1931, 93, 171–180.)—Barber and Kolthoff (*J. Amer. Chem. Soc.*, 1928, 50, 1625; *ANALYST*, 1928, 53, 456) described a method for the quantitative precipitation of sodium with uranyl zinc acetate and the gravimetric determination of the sodium as uranyl zinc sodium acetate. Samples with 2 mgrms. of sodium gave accurate results. Phosphate was precipitated by the reagent, and thus had to be removed first, but its precipitation as uranyl phosphate has not been found satisfactory. Barber and Kolthoff reported the same difficulty, but obtained satisfactory results with known salt solutions after removal of phosphate by precipitation with magnesia mixture. The determination of sodium in biological work is of importance, and the methods at hand are either laborious, expensive, or subject to considerable error, and, therefore, the applicability of the Barber–Kolthoff method to biological material has been studied. The information obtained and modifications introduced have resulted in the development of definite procedures for the determination of

sodium in urine, serum and material high in phosphate and potassium such as whole blood, stool and tissue. These procedures are described in detail. Powdered calcium hydroxide is used for the precipitation of phosphate and found to be satisfactory, within the desired limits of error, and more convenient than the magnesia mixture. It simplifies the reagents required for the procedure. Its addition clarifies cloudy urines and produces no volume change, and thus eliminates the necessity of diluting to known volume the taking of aliquot parts and the quantitative washing of the precipitate. For urines containing protein solid mercuric chloride as precipitation reagent is used, and again volume changes are avoided. The triple sodium salt is precipitated in a stoppered glass filter, and thus the necessity of washing the precipitate on to the filter is eliminated, thereby saving time and reagent. The authors have studied the necessity of ashing urines, and have investigated conditions for the determination of sodium in urines containing relatively very small amounts of this element. Some results obtained with known solutions are given; they show small percentage errors. P. H. P.

**Electrometric Analyses of Solutions of Hypochlorites.** F. Abribat. (*Bull. Soc. Chim.*, 1931, 49-50, 1119-1138.)—The usual procedure was followed; the platinised or polished platinum electrode and the end of the saturated calomel electrode were placed directly in the sample, a stable potential being obtained after 3 to 5 minutes, when an electrometric titration with (e.g. 0.25 N) sulphuric or hydrochloric acid could be carried out. The curves obtained (E.M.F. as ordinate) for commercial solutions of sodium hypochlorite show 5 distinct portions separated by well-defined points of inflexion, which enable the following determinations to be made:—*Total Hypochlorous Acid*.—This is given by the abscissa between the third and fourth points of inflexion, and corresponds with the reaction  $\text{HClO} + \text{HCl} \rightleftharpoons \text{Cl}_2 + \text{H}_2\text{O}$  (E.M.F. 800 to 950 millivolts). The data obtained during the period of liberation of gaseous chlorine are in agreement with the accepted values for the reversible chlorine electrode. *Free Alkali*.—This is given in terms of the acid corresponding with the abscissa of the curve between the origin and the first point of inflexion. *Carbonate and Hypochlorite*.—The abscissa of the second region of the curve corresponds with the transformation of carbonate into bicarbonate, whilst the total abscissa of this and the adjacent (i.e. third) region represents the liberation by the acid of (a) carbon dioxide from the bicarbonate, and (b) hypochlorous acid from the hypochlorite. It is preferable, however, to take the sum of these abscissae as representing (hypochlorite + sodium carbonate), since the two regions concerned are not separated by a very sharp point of inflexion. *Chlorites* are also represented by the second region of the curve, and are, therefore, obtained by a second titration after removal of the carbonate by precipitation with lime. *Free hypochlorous acid* is obtained by difference from the hypochlorite and total hypochlorous acid values. *Chlorides* are determined by direct titration with silver nitrate solution by means of a silver electrode, since the silver salts of chlorous, hypochlorous and (in certain cases) chromic acids are precipitated only at the conclusion of this titration. Alternatively, this type of titration may be carried out,

before and after the reduction by the usual method, with arsenious oxide in the presence of sodium bicarbonate (*cf.* Penot, *J. prakt. Chem.*, 1851, **54**, 59). The method, which is rapid and unaffected by chlorates and perchlorates, was also applied to "chloride of lime," but in this case the results may be upset by the co-existence of two sets of reactions, *viz.* those taking place in the solution and those between the solid and acid liquid phases. It is pointed out that the term "active chlorine" has been applied to (a) the amount of hypochlorite chlorine; (b) the amount of free chlorine producing the same effect as a given weight or volume of a bleaching agent.

J. G.

## Water Analysis.

**"Carbonate Number" in Water Analysis.** O. Mayer. (*Z. Unters. Lebensm.*, 1931, **62**, 271-291.)—The relation between degrees of carbonate hardness ( $x$ ) due to magnesium and calcium, and the total solids ( $y$ ) in grms. per 100 c.c., is given by the ratio  $1000x/y$ , and is known as the "carbonate number." This value serves a useful purpose for the characterisation of certain waters, and also for the detection of "infiltration" into the source of one water, of another having a different carbonate number. Its relation with the other analytical numbers, particularly with the total hardness, free carbon dioxide and total alkalinity, is discussed, and a table shows the relation between carbonate hardness and bound and free carbon dioxide. A rapid scheme of semi-quantitative analysis, based on these considerations, is outlined. Thus, an indication of the alkalinity is obtained by direct titration of 100 c.c. of the water with 0.1 *N* hydrochloric acid containing 5 c.c. of 0.05 per cent. methyl orange solution per litre, the end-point being the appearance of a brownish-red colour. The total hardness may then be found by bleaching the indicator with one drop of bromine water, the solution being boiled for a short time, diluted to 100 c.c. with distilled water, and 0.5 c.c. of a 0.4 per cent. alcoholic solution of phenolphthalein added. Titration with a clear 0.1 *N* solution of sodium palmitate by Blacher's method (Kolthoff, *Die Massanalyse*, 1928) may then be carried out. The gravimetric method should be used for more precise determinations, *viz.* the water is evaporated in a platinum dish, silica removed, the iron and aluminium precipitated with ammonia, and the calcium obtained as oxalate and determined as oxide, and the magnesium as pyrophosphate. "Chalk-aggressive" carbon dioxide is found by determination of the change in alkalinity of 250 c.c. of the filtered water after digestion with finely-powdered, washed marble for 24 hours to 3 days. Numerous examples of the method are given and discussed, and data for 91 waters (Bavaria and Danube valley) are tabulated and discussed with special reference to geological origin.

J. G.

**Determination of Nitrates in Drinking Water.** W. Mulder. (*Pharm. Weekblad*, 1931, **68**, 995-997.)—The conclusion of Scheringa (*id.*, 1930, **67**, 1362) that sodium salicylate gives a stronger colour than phenol-sulphonic acid in this determination, and is otherwise preferable, is confirmed. The correction factor for the colorimeter reading given by Scheringa to be used in the presence of chlorides

[namely  $100/(100-10a)$ , where  $a$  is the chloride present in mgrms.] is shown, however, to be valid only for 2 mgrms. or less of Cl' in the presence of 0.1 mgrm. of nitrate in 10 c.c. of water. Low results are obtained for 3-10 mgrms. of Cl' under these conditions, and the factor 1.25 should then be used. The author, therefore, takes a volume of sample (e.g. 10 c.c.) containing about 0.1 mgrm. of nitrate, adds 5 drops of 2 per cent. sodium chloride and 1 c.c. of 0.5 per cent. sodium salicylate solutions, evaporates to dryness on the water-bath, and adds 1 c.c. of sulphuric acid to the cool residue. After 10 minutes the mixture is washed into a colorimeter tube with 10 c.c. of ammonia or sodium hydroxide solution, and the colour is compared with that of a standard containing exactly 0.1 mgrm. of nitrate and 5 drops of 2 per cent. sodium chloride solution in 10 c.c., the colour being developed by a similar procedure. Accurate results are obtained for 0.1 mgrm. of nitrate in the presence of 0.01 to 0.1 mgrm. of nitrite, but for larger quantities (1 and 2 mgrms.) of the latter, high results are obtained. J. G.

## Physical Methods, Apparatus, etc.

**Chromium Steel Vessels for Analytical Purposes.** A. Krüger. (*Chem. Ztg.*, 1931, 55, 682-683.)—Vessels made from chromium steel (V4A steel) are claimed to be preferable to glass or porcelain ware for certain analytical work where freedom from contamination of solutions by substances, e.g. silica, alumina, etc., which can be dissolved from glass by alkaline liquids, is desired. Two examples, viz. the gravimetric determination of iron and of aluminium by precipitation as hydroxide, have been studied, and good results were obtained. The acid solution was kept in a glass vessel and poured into the stainless steel vessel containing an excess of ammonia, in which, in the determination of aluminium, the subsequent adjustment of the solution to feeble alkalinity for the precipitation was made. In tests of the resistance of chromium steel vessels to various solutions, hot dilute ammonia was found to be without action; but the vessels suffered slight attack by stronger alkaline solutions. The results are summarised in the subjoined table.

Liquid.	Temperature.	Time.	Test with sodium sulphide.
KOH (1 per cent.)	100° C.	20 minutes	Colourless
KOH (5 per cent.)	100° C.	20 "	Very slight yellow
KOH (10 per cent.)	100° C.	20 "	Slight yellow
KOH (1 per cent.) + H <sub>2</sub> O <sub>2</sub>	70° C.	20 "	Colourless
KOH (1 per cent.) + Na <sub>2</sub> S	70° C.	20 "	Clear, colourless
KOH (5 per cent.) + H <sub>2</sub> O <sub>2</sub>	70° C.	20 "	Very slight yellow
K <sub>2</sub> CO <sub>3</sub> (1 per cent.)	100° C.	20 "	Colourless
K <sub>2</sub> CO <sub>3</sub> (5 per cent.)	100° C.	20 "	Very slight green
K <sub>2</sub> CO <sub>3</sub> (10 per cent.)	100° C.	20 "	Slight green
NH <sub>3</sub> (5 per cent.)	70° C.	20 "	Colourless
NH <sub>4</sub> Cl (3 per cent.) + Na <sub>2</sub> S (2 per cent.)	70° C.	20 "	Clear, colourless
Ba(OH) <sub>2</sub> (0.5 per cent.)	100° C.	20 "	Colourless

(In these tests, 200 c.c. of liquid were used; this was tested for dissolved iron, after being in the vessel for the stated period, by adding sodium sulphide solution; "slight green" and "very slight green" was found from special experiments to correspond with the solution of 0.2 to 0.1 mgrm. of iron.) It is concluded that the



choice between a glass or stainless steel vessel for a particular analytical determination involving alkaline liquids will depend on which is the lesser of two evils: contamination by silica, etc., from the glass, or by iron from the chromium steel. (Cf. G. A. Stokes, *ANALYST*, 1929, 54, 538.) S. G. C.

## Reviews.

CHEMISTRY, FLAVOURING AND MANUFACTURE OF CHOCOLATE, CONFECTIONERY AND COCOA. By R. H. JENSEN, M.Sc., F.I.C. Pp. x + 406. J. & A. Churchill, 1931. Price 27s.

The author of this book has been so singularly successful in condensing into the small space of one volume such a mass of information and experience that it is impossible adequately to review the work within the space allotted; all that can be done is to indicate the scope of the work and to refer in more detail to certain sections.

The raw material, cocoa, forms the subject of the first chapter, in which the types and commercial varieties are dealt with briefly and concisely. The nature of the changes which occur during the fermentation of the bean has been widely studied, and a résumé of this shows only too clearly that there is a long path to travel before we determine to what substances the cocoa aroma is due. The statistics of prices and production and the economics of the industry are useful additions not usually found in a work of this kind. The value of cocoa and cocoa products as a food, a fact which is more recognised abroad than in this country, has been given due prominence. It is doubtful whether sufficient notice has been taken of the work of R. O. Neumann on the digestion of the proteins, which, though now somewhat old, is still convincing.

Manufacturing processes are described minutely and a critical examination is made of a number of modern machines and appliances. Right through this section, as indeed through the whole book, the chemical and physical aspects are considered and explained, use being made of carefully selected data, in many cases hitherto unpublished. A criticism of some of the illustrations might be that, while they depict plant well known to those in the industry, they do not give much indication to the layman of their *modus operandi*.

A consideration of chocolate in the molten state shows it to be composed of finely divided particles of cellular and non-cellular structure, the solid phase suspended in a fatty fluid, the liquid phase. The behaviour of this magma during solidification determines the appearance, texture, and stability of the product. Particle size of the component parts of the solid phase requires measurement if conclusions are to be drawn as to the surface to be covered by the liquid phase. The methods for making these determinations are described. As regards the liquid phase, in recent years much work has been done on the behaviour of mixed glycerides during changes of state, and these the author has collated in an excellent chapter on confectionery fats. The significance of the solidification test

and of dilatometer measurements is rightly emphasised. The author has standardised an apparatus and minutely described the procedure for the former, a most essential factor omitted by other investigators, and, using the latter, gives interesting data from his own experiments, which go a long way towards explaining some of the abstruse phenomena occurring in manufacturing practice.

In the sections devoted to confectionery and the ingredients used therein, a mass of analytical data has been employed not only to evaluate these but to explain their properties and how to use them in order to obtain the desired results. Here, as elsewhere in the book, the author is handicapped in that he is not free to use and discuss much important and pertinent work which has been carried out by the British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades.

A wide survey of flavouring materials and their properties fills a section, and is preceded by a chapter on the principles of flavouring, which, though somewhat dogmatic, indicates factors which must be taken into consideration in the successful use of flavouring materials. A few useful pages at the end are devoted to a summary of legal standards for cocoa and chocolate in various countries. The work shows the extent to which chemical and physical sciences, not to mention dietetics and physiology, enter into the manufacture of what is not only a delicacy, but a food stuff. The scope of the chemist's work is clearly indicated, and shows the way in which the value of his work may be most beneficial to the business.

The author is to be congratulated upon the production of a book which contains up-to-date knowledge on many points and is destined to take a very prominent place in the literature of this industry. B. G. McLELLAN.

LUNGE AND KEANE'S TECHNICAL METHODS OF CHEMICAL ANALYSIS. 2nd Edition, edited by the late CHAS. A. KEANE, D.Sc., Ph.D., and P. C. L. THORNE, M.A., M.Sc., Ph.D. Volume III. Pp. 698. London: Gurney & Jackson. 1931. Price £3 3s.

The progress of research in the different branches of chemical technology covered by the articles in this volume has been very different, so that some of the articles are new work rather than revision, whilst others show comparatively little alteration from those which they supersede.

The article on Clays, Ceramic Products, and Refractories, now divided into two portions—chemical examination and physical examination—comes under the first head. The chemical portion is written by Mr. H. V. Thompson, M.A., F.I.C., and the physical portion by Mr. S. R. Hind, B.Sc., A.R.C.Sc., F.I.C., both of them on the staff of the North Staffordshire Technical College, and each of them has carried out his task most satisfactorily. The methods of chemical analysis cover fireclays and silica rocks and their products, felspars, bauxites, limestones and dolomites, barytes, zirconia, chromite, fluorides and glazes; and though there is little that is new, yet all the methods are described with great clearness and conciseness, and details of manipulation, where attention to those details is necessary to success, are carefully and minutely given. The growth in importance of the

examination of physical characters is indicated by the greatly increased space devoted to this part of the subject. Specific gravity, true and apparent; grading and determination of particle sizes; adsorption and viscosity; plasticity, binding power, and shrinkage of raw clays; thermal expansion, refractoriness, and deformation under load; porosity; resistance to stresses and abrasion; dielectric strength: all these, and other related properties, are considered, and methods—many of which have been worked out in the Stoke College—are given for their determination or estimation. All are well and clearly described, and comprehension is aided by many diagrams and illustrations of apparatus. The whole forms a monograph of great merit, and will become almost indispensable to those who have to deal with these materials.

The same may be said of the Section on Glass, which has expanded from 37 pages in the old edition to 150 pages in this. Mr. J. D. Cauwood, M.Sc., of the Canning Town Glassworks, and Mr. J. H. Davidson, M.Sc., of Messrs. C. E. Ramsden & Co., have collaborated here, and have produced a work which everywhere bears the impress of minds and hands engaged in the industry of which they treat. Valuable features of this Section are, first, short accounts of raw materials and their impurities, the uses of the raw materials and the effects, on these uses, of the impurities; and second, tables giving the average composition of various types of glass. If works on analytical chemistry, especially of industrial materials and products, were not so strictly confined to the description of methods, but gave a little trustworthy information of this kind, it would be all to the good. It would be tedious to enumerate in detail the contents of this section; enough to say that methods are given for the analysis of all raw materials and of every variety of glass, and for the examination into the durability of glasses and their attack by chemical and other agencies; that the descriptions are almost uniformly good, concise, and clear; and that the authors have evidently tested even the most recent work, and have here embodied such of it as has met with their approval.

The Section on Calcareous Cements, by Dr. Geoffrey Martin and Mr. E. A. Bowes, covers the analyses of limestones, limes, lime-sand mortars, and natural and artificial hydraulic cements; and, like the previous article, it not only gives methods, but furnishes useful relevant information regarding hydraulic limes and natural cements, with typical analyses showing their composition. Not only the chemical analyses, but mechanical and physical tests are described. The analyst dealing with these substances will find all he wants here, though the descriptions are not all on the same high level as those in the first two sections: there is occasional loose phraseology, and there are slips which do not seem to be all "typographical." We find Warington misspelt on p. 287, "bulk" for "burette" on p. 297, and a wrong sign inside the brackets in the calculations near the top of p. 294; and a large part of the description of the Southard viscometer on pp. 312–314 is absolute and almost verbal repetition. We have also phrases like "Between each test" and "Between zero time to the point . . .", and the authors' fondness for that frequent trap "the latter," leads them to direct, on p. 322, that a glass plate should be placed on the trowel used, instead of on the mould. A little

more thought given to things like these might have greatly improved the article and helped the reader; but, in spite of these shortcomings, it is a valuable and useful contribution.

Drinking Water and Water Supplies, and also Sewage and Effluents, are treated in separate sections, by Dr. Gilbert Fowler. The physical, chemical, microscopical, and bacteriological examination of water are all dealt with, the chief part of the work being, naturally, concerned with the chemical examination. Dr. Fowler prefaces the chemical portion with the statement that the results are most conveniently expressed in parts per 100,000; but he himself apparently scorns convenience, for nearly all the figures given in the section are either "milligrams per litre" or "parts per million." The methods given cover the usual course, though modern developments have brought the need for detecting small quantities of free chlorine (for which the American Public Health Association's tolidine coloration, method is given), and of iodides—here Hunter and Brubaker's method might have been described. As the action of waters on lead is discussed, it seems a pity that Dr. Houston's very convenient method of estimating lead-solvency is not given. The methods for nitrates, and for determining gases in water, are fully and clearly described, and the whole section fulfils its purpose well.

The short article on Feed Water for Boilers, by Mr. L. O. Newton, of Sofnol Limited, confines itself to a description of methods of determining the mineral constituents of the water and its hardness, and is all very concise and clear.

The Section on Sewage and Effluents gives directions for sampling and preliminary examination, and for determination of dissolved solids, suspended and colloidal matters, oxygen absorption, alkalinity or acidity, nitrogen in its various forms, sulphides, chlorine, individual organic substances, coal gas products, and tests for putrescibility. These are followed by a short but valuable discussion on the criteria of contamination due to effluents, and their injurious effect.

Sections on Fertilisers and Feeding Stuffs follow, from the pen of Dr. Bernard Dyer. These form, to all intents and purposes, a new treatise. Much of the work is, of course, conditioned by the requirements of the Fertilisers and Feeding Stuffs Act, but the wealth of Dr. Dyer's experience crops out continually in the details of the methods of analysis given, and in the discussion of their applicability in various circumstances; such a description of the Kjeldahl process, for example, as is here given, is not to be found elsewhere. The account of calcium cyanamide, too, is very full and clear. Not only the official methods for Feeding Stuffs under the Act, but also those of the International Committee, are given, and the article ends with useful paragraphs on the detection of mustard, hydrocyanic acid, and castor oil seed in feeding stuffs.

"Soils" are dealt with by Sir Daniel Hall, who gives an account of the derivation and characters of different soils before going on to their sampling, mechanical examination, and chemical examination. Methods are given for nitrogen, soluble mineral constituents, carbonates, nitrates, pH value, lime requirement, and "exchangeable bases." The methods are not new, of course, but their collection here in one treatise is useful. There occur two or three slips (as it seems to me),

in description. On p. 592 we treat 20 grms. of soil and make the solution up to 250 c.c.; this is spoken of as a 10 per cent. solution. On the same page, in determining potassium, it is surely a strange direction to wash the platinum precipitate and excess platinum chloride on to a filter with ammonium chloride. And on p. 599 I do not follow the direction to deduct the results of the second litre of leachings from those given by the first.

The last Section, on Air, is written by Dr. J. S. Owens and Mr. J. H. Coste, and is entirely new. It describes methods for the determination, not only of the ordinary constituents of fresh air, but of impurities of various kinds which may occur locally in greater or smaller amount—usually in very small proportions—derived from the industrial and other operations of civilised life: carbon monoxide, nitrogen oxides, ozone, sulphur compounds, and various organic vapours; and it deals also with the suspended matter in the air and the various appliances in use—largely due to Dr. Owens—for its examination.

A review of a huge and detailed work like this, unless it is to be of the dimensions of a treatise, can be little more than a summary of the contents. Detailed criticism would swell it to intolerable dimensions, and, on the whole, little criticism is called for—the work worthily maintains the tradition of its predecessors, and yet represents the present-day position. There is, however, one general criticism, applicable in very differing degree to different sections in the volume, that I should like to offer. It is, perhaps, a matter of personal preference, and not to be decided absolutely, whether directions for analytical processes should be in the imperative mood or in the passive voice of the indicative. I go heartily for the imperative, but would blame no man for using the other. But we have, I think, a right to ask for consistency; it is to me, and I expect to others, very disturbing to read "Cool and add 150 c.c. of hydrochloric acid: the contents are now stirred and filtered: wash three times with water: the filter paper is now removed"; and so on. Many examples of this will be found throughout this volume, and I think one should protest against it.

Take it for all in all, this is a collection of excellent treatises, in which he who consults them will seldom, if ever, fail to find what he seeks, and which should be in the library of every analyst who has to do with any of the subjects of which it treats.

J. T. DUNN.

**BACTERIOLOGICAL TECHNIQUE.** By J. W. H. EYRE, M.D., M.S., Professor of Bacteriology in the University of London. Third Edition. Pp. xii+619, with 238 figures in the text. London: Baillière, Tindall & Cox. 1930. Price 21s.

A perfunctory survey of this book leaves the impression that little except hydrogen ions has disturbed the placidity of the routine in bacteriological laboratories since the publication of the second edition in 1913. However, the truth is that the book has been thoroughly revised, but so unobtrusive are the additions, numerous though they be, that only comparison, page by page, with the preceding volume reveals them. The Preface is useless as an indication of the progress of the book.

Great improvement in the illustrations is shown. Many of the old cuts have been replaced by diagrammatic sketches, and there are twenty-two new pictures of the microscope and accessories.

Newly described apparatus includes a reference to the electrical hot-air steriliser and the modern innovations in bacterial filters, namely, the Seitz asbestos, the plaster of Paris, and the collodion ultra-filters, are described, as also is the air-pressure method for testing porcelain candle filters.

So large a variety of stains was evolved at an early date in bacteriological history that no surprise is felt that only five additions are thought necessary. They are Czapelewski's carbol-fuchsin, Fontana's method, Murray's iron alum haematoxylin, van Giesen's Nile blue, and the Barnard Topley method for demonstrating flagella during life.

Additional media are more numerous: alkaline egg broth, alkaline egg agar, Robertson's cooked meat medium, five of the war-time "trypsin digests" media, Thornton's standardised agar, Wilson and Blair's G.I.B.S. agar, Russell's double sugar agar (coloured with phenol red as an alternative to litmus), Koser's citrate broth for *B. coli*, Douglas's tellurite serum for *B. diphtheriae*, two of the media devised by Flemming for the haemophilic bacilli, and five new media for protozoa.

Eight pages are devoted to new devices for achieving anaerobiosis and reduced oxygen atmosphere in cultures, and two methods for micro-selection are described.

It is only fair that it should be left to the deviser of Eyre's scale to publish coloured diagrams showing the very great difference between the pinkish phenolphthalein end-point of the Eyre scale and the deep magenta to which the American Committee of Bacteriologists take their titration—a source of confusion all too little known.

Bacterial haemolysis and the bacteriophage are given attention, and acid production by bacteria now means another task for the comparator.

At a time when many are trying to classify bacteria, and there is an understandable over-eagerness to support something with plausible claims to notice, it is refreshing to find Professor Eyre summing-up that: "no really successful classification of the Schizomycetes has yet been drawn up, despite the elaborate system of families, tribes, and genera promulgated by the Society of American Bacteriologists in 1920, and the varying morphological characters of the Schizomycetes still continue to be utilised as a basis for classification, as they were by Baumgarten in 1890 and Sternberg a few years later."

Many processes of sanitary utility receive detailed notice, and alternative methods are often described. Two methods for the sanitary examination of shell-fish: that devised by Houston, and that of Klein which has been adopted by the Worshipful Company of Fishmongers for the control of retail trade, are mentioned. For disinfectants, the author describes at length the process he favours in which he now incorporates some of the details of *The Lancet* (1909) process. He also refers to the Hygienic Laboratory (U.S.A.) method. The Rideal-Walker method is dismissed in a few lines, which is scant justice to a device that, more than any other, had made the standardisation of disinfectants practical by recognising the

many circumstances that disturb the action of disinfectants on naked bacteria. It is still the method most frequently adopted, not only by commercial houses, but by Government Departments. The Lister Institute method is not mentioned, in spite of the degree of popularity it has received. Much could be written in favour of Eyre's method, but the others are in equal demand and worth more than perfunctory notice.

Milk examination includes both Breed's "microscopical count" and Frost's "little plate method," but it ignores the starvation method of enumerating bacteria approved in "Memo. 139/Foods" for Graded Milk (ANALYST, 1929, 54, 235).

Misprints include "nutose" for "nutrose" (inset p. 512), and "tinige" for "tinge" (p. 161), while a formula of  $\text{CaOS}_4$  (p. 51) and the substance "piridin" (p. 18) are not serious. But for an author having an enviable acquaintance with his science and a profound knowledge of its applications to convey the impression that nothing is good unless German, can only signify the perpetuation of pre-war material. Witte peptone did good service for many years, but when, in 1930, it is prescribed (no alternative being allowed) for nutrient broth, lemco broth, peptone water, and even for the bile salt broths, it would appear that fair trial of modern English peptones has not been made, and that the disconcerting attributes of some former English preparations have unfairly prejudiced the entirely (I believe) satisfactory modern manufactures. English-made stains get little more appreciation: thus, on p. 102, "The stains employed should be those prepared" by two named German firms, and a paltry footnote mentions that an English firm prepares "a limited variety of reliable stains." The totally unnecessary exaltation of things German extends even to words; thus, while everywhere else a "loop" needle is called a "loop," we find here the information (p. 83) that it "is termed a loop or an oese." A footnote that points out with no evasion the advantage which chrome-nickel wire has over platinum will please all.

The modifications of the Romanowsky stains (those of Jenner and Leishman are mentioned, whilst Giemsa stain, popular though it be, is not) are given a restricted sphere of blood-, and protozoa-staining, whereas they give illuminating information on pus films, milk sediments and many other preparations.

It is probably unintended, but may be seriously misunderstood when the routine scheme for the bacteriological examination of a water includes (p. 512) the following direction: "Determine the pathogenicity for mice (subcutaneous inoculation) and rabbits (intravenous inoculation) of the streptococci isolated." Only in a very exceptional case could the results have any significance.

All who have used this book appreciate it, but it is often involved in technique where simplicity is needed; and, though the little tips that lessen labour and smooth difficulties are well to the fore, these are largely original. There are hosts of others in books, English and American, that should be introduced if this book is to do the best service to bacteriology. At present, Professor Eyre has the field of bacteriological technique to himself, and did he but realise the worry his directions can cause, as well as the godsend he knows the book to be, he would have scrapped

much and re-written more. He would also have given us a less heavy book by remodelling binding, paper and type.

WILLIAM PARTRIDGE.

**DAIRY BACTERIOLOGY.** By Prof. ORLA-JENSEN. Translated by P. S. Arup. Second Edition. Pp. x+198. London: J. & A. Churchill. 1931. Price 18s. net.

This book is a second and revised edition of Prof. Orla-Jensen's well-known work on a subject of which he is an acknowledged master.

The author has shown much wisdom in the treatment of the subject, in that he has not attempted to write a treatise on bacteriology and on the science of dairying, but has arrived at a happy blend of both. To secure the maximum benefit, the reader must possess a knowledge of both subjects, and the student will require the guidance of an expert. The book has been most admirably translated by Mr. Arup, and is well indexed.

The subject has been dealt with in two parts: Part I, which comprises about one-third of the book, deals with elementary bacteriology, and is splendidly illustrated. To the bacteriologist, parts of the classification and nomenclature are confusing, since they do not conform to any of the widely accepted standards.

The method detailed for milk examination appears likely to increase the inevitable errors of dilution and would not be applicable generally to English clean milk.

A serious error is found on p. 45, where organisms of the *aerogenes-proteus* type are said to form spores. They are, of course, not spore formers.

Part II, which comprises the greater part of the book, is devoted to the scientific control of milk and milk-products, the making and control of starters, and the ripening of cheese. The causes of the various faults encountered in raw and manufactured milk are adequately treated.

In the section devoted to clean milk production the English reader will find several statements with which he cannot agree. For example, on p. 64, it is stated that "Dry milking . . . is extremely difficult to carry out in practice; it is facilitated by smearing the teats with a little vaseline or fatty material." Apart from aesthetic consideration, this practice has been found to be unnecessary in England. It appears from the paragraph at the top of p. 65 that the author is not conversant with the progress which has been made in the efficiency and hygienic use of milking machines in England and America.

It is very astonishing to one accustomed to "clean milk," as understood in England, to learn, on p. 72, that it is advisable to freeze milk if it is to be kept longer than 24 hours; there is ample evidence that the numbers of bacteria in clean milk remain very small even after 24 hours at temperatures of 15° C. and over.

With the reservation that the reader will do well to recognise that the English and Danish ideas on clean milk are different, this book can be heartily recommended to the reader, who will profit by Prof. Orla-Jensen's unique knowledge of the application of the science of bacteriology to the dairy industry.

A. T. R. MATTICK.



**INTERNATIONAL REGISTER OF SPAS AND MEDICINAL WATERS.** Published by the International Society of Medical Hydrology, 55, Wellington Road, London, N.W.8. Price 1s.

The object of the Register is to afford a full record of the characters, chemical, physical, biological, and therapeutical, of the medicinal waters of natural origin, such as occur in the well-known spas, and it is hoped later to deal with other media, such as sea-water, muds, peats, etc., which are now used in hydrological practice. It has been compiled by the Standard Measurements Committee of the International Society of Medical Hydrology, which was founded in 1921 to promote the study of Medical Hydrology and to form an International Union of Scientific Workers in this branch of medicine.

The immediate aim of the present publication is to aid the adoption, throughout the world, of uniform methods of examination (by analysis, etc.), and of recording the various data, which may enable medical practitioners and others to understand and compare the properties and therapeutic values of the waters throughout the world.

The adoption of standardised methods and of uniformity of expression is, of course, essential in an international publication, and the general plan of the Register should go far to ensure this, but the explanatory paragraphs can hardly be regarded as masterpieces of English prose.

Neither brevity nor clarity is their strong point. Some of the statements are too obvious to require publication, and in others the phraseology is, at its best, awkward, and at its worst barely intelligible.

What, for instance, is the meaning of the reference (p. 3, lines 2 and 3 of the last paragraph) to the biological properties of waters relative to their effect on "vegetable and animal life," or (on p. 7, lines 10 and 11), how does the method of combining the ions so as to form salts (or, as the Register calls them, "salines") "represent" the "physical state" of the water? As a final example, on p. 9 (at the top of the page) are set forth some of the directions of the International Committee, as follows: "(a) To allow to appear in the Register only such data as are satisfactory," etc. "(b) To reject all data which are not satisfactory." Surely these paragraphs are identical in meaning, obvious and unnecessary.

These, and other eccentricities of diction are, no doubt, largely the product of a committee whose members are of different nationalities, but it is particularly unfortunate that the first edition of the Register should suffer in this way. However, the only aim of these criticisms is to be helpful, and it is fair to add that the specimen report on pp. 17-20 clears up most of the doubts and uncertainties in the general text.

A wide tolerance as regards methods of stating analytical results is natural, and perhaps unavoidable, at this early stage of the scheme, but it is hoped that before long one uniform plan can be adopted. Unfortunately, even in this country, we are still far from unanimous, and this International Register may point the way.

The Society is to be congratulated on initiating a scheme which is as much needed as it will eventually be valuable to all concerned. C. H. CRIBB.



# THE ANALYST

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## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS.

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AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, November 4th, the President, Dr. J. T. Dunn, being in the chair.

Certificates were read for the first time in favour of:—Thomas Whittaker Lovett and William Charles Alfred Wise, B.Sc.

Certificates were read for the second time in favour of:—Charles Hubert Francis Fuller, B.Sc., F.I.C., Ganesh Chandra Moitra, B.Sc., Eric Charles Wood, B.Sc., A.R.C.S., and Robinson Pearson Wood, M.Sc., F.I.C.

The following were elected Members of the Society:—Raymond Merefield Edwards, B.Sc., Llewelyn John Howells, B.Sc., Donald Neil McArthur, D.Sc., Ph.D., F.I.C., F.R.S.E., James Sword, M.A., B.Sc., Ph.D., A.I.C.

The following papers were read and discussed:—"A Reliable Method for the Quantitative Separation of Titanium from Tantalum and Niobium," by W. R. Schoeller, Ph.D., and C. Jahn; "The Separation of the Earth Acids from Metals of the Hydrogen Sulphide Group," by E. F. Waterhouse and W. R. Schoeller, Ph.D.; "Evaluation of the Menthone Content of Peppermint Oil," by J. Reilly, M.A., D.Sc., F.R.C.Sc.I., N. Noonan, M.Sc., and P. J. Drumm, Ph.D.; "The Determination of Vanillin in Chocolate and Cocoa Butter," by D. M. Freeland, A.I.C.; and "The Direct Determination of Nitrogen in Gases," by H. R. Ambler, B.Sc., A.I.C.

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## Fat in Shredded Suet.

THE following resolution regarding the minimum fat standard for shredded suet was passed by the Committee of Public Analysts and confirmed by the Council:

"It is the opinion of this Committee that, pending the establishment of any legally authorised standard, shredded suet should contain not less than 83 per cent. of fat. A lower percentage of fat should be regarded as indicative of excess of flour. This fat standard is sufficiently low to cover any reasonable variation due to uneven distribution of flour, and is to be considered as an absolute minimum standard. The Committee adheres to the opinion that the nature of any admixture to suet should be declared."

## Death.

WE deeply regret to record the death, on November 12th, of WILLIAM FOULKES LOWE at the age of 81. An obituary notice will appear in a subsequent issue.

## The Physico-Chemical Constitution of Milk Powder.

BY L. H. LAMPITT, D.Sc., F.I.C., AND J. H. BUSHILL, M.Sc., A.I.C.

(Read at the Meeting, February 4th, 1931.)

### SOLUBILITY CHANGES.

I. INTRODUCTION.—One of the most important properties of spray-dried milk powder is its solubility,\* which in a powder of high quality reaches approximately 100 per cent. Certain factors (not concerned with the manufacture itself), however, have an adverse effect on solubility, and knowledge and evaluation of such factors are obviously desirable. The deleterious effect of moisture has been observed by other workers, but little detailed systematic work, so far as we have been able to trace, has been reported dealing with the effect of moisture considered in relation to the factors time and temperature.

We have already demonstrated<sup>1</sup> that the changes which take place in spray-dried milk powder on absorption of water are of a complicated nature; *inter alia*, that at a minimum moisture content (which we have called the Critical Moisture Content) the lactose crystallises, and fat, previously not available for solution in organic solvents, becomes free. Moreover, this moisture content is proportional to the solids-not-fat of the powder. Concurrently with that study we have concerned ourselves with the solubility changes, and, although it appears that finality

\* We use this term in the usual sense applied to milk powders; it includes the true solubility of certain constituents and the colloidal dispersion of others.

in the evaluation of the effects of all the factors concerned is not in sight, certain definite results have been achieved. The present paper shows the peculiar difference between milk powders made by roller processes and those made by spray processes in respect of the absorption of moisture, describes the methods of determining solubility with due regard to the effect of the fat, and indicates the distribution of the proteins between the soluble and insoluble fractions of spray-dried milk powder.

**II. METHODS OF PREPARATION OF MILK POWDERS OF DIFFERENT MOISTURE CONTENTS.**—It will be appreciated that before a study of the effect of moisture absorption on milk powder could be carried out, definite methods of controlling this absorption and of determining the moisture content itself had to be evolved. An initial difficulty, and one which had not been anticipated (it apparently has not been experienced by previous workers), was to obtain a homogeneous mass of milk powder throughout which the moisture content was uniform. The method originally employed was to expose a layer of the powder ( $\frac{1}{4}$ " to  $\frac{1}{2}$ " thick) to a humid atmosphere with periodic stirring and determinations of moisture. This method, however, was found to produce a product of appreciable moisture variation; powders containing up to 6 per cent. moisture showed a variation of about 0.1–0.2 per cent., while, as the moisture increased to 9–10 per cent., the difference between maximum and minimum figures was about 1.5 per cent.

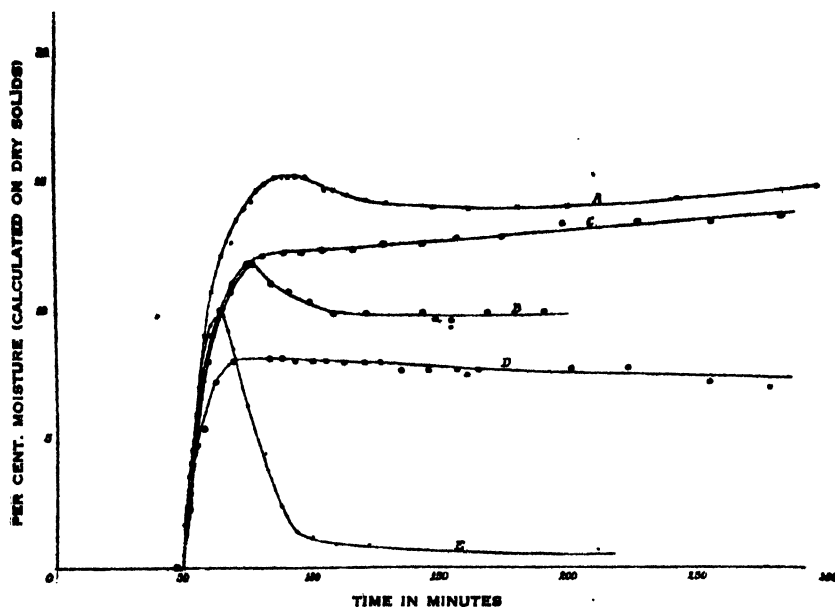
It seemed desirable, therefore, that the actual absorption of moisture should be studied in some detail, and a method, using a layer of powder of the order of one particle in thickness, was employed, the powder (0.02 grm. to 0.20 grm.) being spread on the pan (40 sq.cm. aluminium foil) of a delicate torsion balance.\* The change in weight of the powder, when placed in atmospheres of different humidities, is recorded by measuring, with a travelling microscope, the movement of the arm carrying the pan.

It is not proposed to discuss here these results, except in so far as they give some explanation of the difficulty of obtaining homogeneous samples of a milk powder which has been exposed to a moist atmosphere. Graph I shows the type of results obtained with four samples of milk powder, all of different brands, curves A and B being for spray-dried powders and curves C and D for roller-dried powders; curve E illustrates the results obtained with spray-dried lactose.\* The particular point to be noticed is that while the roller-process powder gives regular curves, spray-process powders, before reaching equilibrium with the humidity of the atmosphere, show a definite maximum followed by a subsequent loss. This phenomenon is explained by the lactose curve, where absorption of moisture up to about 10 per cent. is followed by an almost complete loss of the whole of the moisture.† Another point of interest in this connection is that the lactose of spray-dried milk powder is amorphous and optically inactive, whereas that of roller process powder—as far as our experience goes—is crystalline. It may be remarked

\* Spray-dried lactose (prepared on an experimental drying plant) containing approximately 1 per cent. moisture, as determined by the Mojonner vacuum oven method.

† This peculiarity of spray-dried lactose is not common to all forms of lactose, for tests have shown that the  $\alpha$  hydrate,  $\alpha$  and  $\beta$  anhydrides are all normal in their absorption of water.

that the curves obtained for moisture absorption of spray-dried milk powder explain in part the phenomenon noted by Supplee,<sup>3</sup> namely, that milk powder exposed to an atmosphere of between 50 per cent. and 70 per cent. humidity first gains and then loses in weight. Supplee apparently found this to be common to all milk powders "irrespective of the method of manufacture," but Graph I indicates that the phenomenon is restricted to the spray-dried product.



GRAPH I.

The rate of moisture absorption (as determined by a torsion balance) of different powders over water at 25° C. in a closed container.

Curve A.	Spray-process milk powder	(1)
" B.	" " "	(2)
" C.	Roller-process milk powder	(1)
" D.	" " "	(2)
" E.	Spray-dried lactose	"

We are of opinion, therefore, that the difficulties found by us in obtaining a homogeneous powder of high moisture content can be ascribed to this cause, namely, that the processes of absorbing and losing moisture may be going on concurrently, owing to specialised local conditions.

It appeared, therefore, that no great improvement in the original method of experimentation could be achieved, but nevertheless an attempt was made to simulate on a large scale the actual conditions of the torsion balance experiments.

Spray-process milk powder was puffed on to glass plates approximately 3 ft. square, and then placed in a room (at ordinary temperature) adjusted to some known humidity. After a definite time the whole of the powder was collected, and as many moisture determinations were made as the amount of powder would

allow. Only a few results are given below in Table I, but they illustrate the variation likely to be obtained under such conditions.

The Mojonner vacuum oven method<sup>4,5</sup> was employed for the determination of moisture throughout this work; the results are calculated as a percentage on the dry solids present, to make them comparable with the figures given in a previous publication.<sup>1</sup>

TABLE I.

	Relative humidity. Per Cent.	Time of exposure. Hours.	Moisture. Per Cent.	Range of variation.
Separated milk powder ..	68	17	8.34, 8.58, 8.43, 8.10, 8.40, 8.41, 8.40, 8.50, 8.36, 8.43	8.10-8.58
Separated milk powder ..	80	23	9.63, 9.70, 9.51, 10.10, 9.70, 9.37, 10.39, 10.02, 9.51	9.37-10.39
Full cream milk powder ..	83	3½	8.65, 8.15, 8.91, 8.54, 8.72	8.15-8.91

These experiments indicated that the moisture determinations, even when the powder was exposed as a thin layer, were liable to variations of 0.4 per cent. when the moisture content was below the critical moisture content, and that such variation may be as high as 1 per cent. when above the critical moisture content.

Since these results were little, if at all, better than those obtained by the simpler method mentioned above, that method was finally adopted.

Other experiments were carried out in which moisture contents were determined after varying periods of time. These confirmed the results obtained with the torsion balance, in that they showed an increase, followed by a decrease, in moisture content when spray-process milk powder was used.

III. METHODS OF DETERMINING SOLUBILITY.—The methods of determining solubility of milk powders may be divided into two main classes, *viz.* (a) those in which the amount of soluble material which dissolves on "reconstructing" is estimated, and (b) those in which the insoluble material under similar conditions is determined.

The methods of Hunziker,<sup>6</sup> Supplee and Bellis,<sup>7</sup> and that of the Government Laboratory<sup>8</sup> fall into the first class. In these methods the milk is reconstructed with warm water (temperature specified in each case), centrifuged or filtered, and either the amount of total solids or the nitrogen content of the liquid is determined.

It appears evident that these methods are suitable for powders of appreciable insolubility, but are not suitable for detecting small differences in powders where the soluble portion is of the order of 99.5 per cent.

The methods of Marquardt<sup>9</sup> and Miyawaki<sup>10</sup> fall into the second class. While these workers appreciate that a determination of the insoluble (rather than

the soluble) fraction is preferable, they wash the insoluble fraction with an unlimited volume of water, and then dry and weigh. These methods determine, therefore, the solubility or the insolubility of milk powder in an unknown large volume of water.

We have, therefore, used a method described by one of us and Hughes,<sup>11</sup> which, in its original form, has lately been advocated by the Standards Committee of the American Dry Milk Institute.<sup>12</sup>

The method consists essentially in shaking a weighed amount of powder with water at 20° C. for 3 minutes in a conical flask, and transferring to a centrifuge tube, finally rinsing out the flask with a small volume of water. After centrifuging, the fat layer is discarded, the supernatant liquid removed, its solids content determined, and the insoluble residue weighed, before and after drying. The weight of the dry residue is then corrected for the soluble solids present, and, from the weight of the insoluble residue thus obtained, the solubility is calculated.

In the work here reported it was found that the powders of low solubility frothed very badly, thereby causing difficulty in transferring the solution from the flask to the centrifuge tube. This was overcome, with satisfactory results, by substituting a 100 c.c. centrifuge tube for the conical flask, whereby the necessity for transference was obviated.

While this method is quite satisfactory for the determination of the solubility of milk powders as such, certain refinements were necessary for the present investigation. In the method as described, the fat in full-cream powders which rises to the surface on centrifuging is removed, and quite rightly not considered to be an insoluble constituent of the powder, whilst the fat remaining emulsified with the supernatant liquid is corrected for in the residue at the bottom of the centrifuge tube, but any extra fat which is taken down by the insoluble residue is considered as insoluble residue.

In an examination of the change of solubility of milk powders it is evident that any appreciable variation in the amount of fat carried down would cause irregularities in the results, thereby vitiating any conclusions which might be drawn.

Since it was found that the insoluble residue of full-cream milk powder contained approximately 40 per cent. of fat, it was evident that for such powders a better idea of the change of solubility would be obtained if it were determined as insoluble solids-not-fat calculated on total solids-not-fat. We term such "Solubility corrected for fat." This necessitated two determinations in addition to those of the method previously mentioned: (a) the fat content of the supernatant liquid after centrifuging; (b) the fat content of the insoluble residue. This latter was carried out as follows:—

The residue (containing the insoluble residue and some soluble residue) after treating with alcohol, drying in a nickel dish and weighing, was transferred to a dry corked tube, and the fat content was subsequently determined by the Mojonnier method. The weight of the residue was approximately 0.4 gm. in most cases investigated in this section. Two quantities of approximately 0.2 gm. each were

weighed into small weighing bottles, 1.5 c.c. of ammonia (sp. gr. 0.880) added, together with about 1 c.c. of water, and the mixture was allowed to stand overnight. The mixture was then washed into a Mojonnier extraction flask, and the determination of fat was made in the usual way.

In the calculation of the solubility, the residue is corrected first for the soluble solids-not-fat in the supernatant liquid, and then for its fat content. The insoluble residue so determined is calculated on the solids-not-fat of the original powder.

IV. TRACING THE CHANGE IN SOLUBILITY WITH TIME, USING POWDERS OF DIFFERENT MOISTURE CONTENTS.—To obtain powders of varying moisture content, spray-dried, full-cream and separated milk powders were spread in layers,  $\frac{1}{4}$ " and  $\frac{1}{8}$ " deep, on flat dishes which stood in large enamelled iron photographic dishes containing water. These were covered by glass plates and allowed to stand at room temperature. At intervals of 15 to 20 minutes the powder was well stirred by hand. At the end of each day's treatment (8 hours), and, whenever a sample was required, the whole of the powder was placed in a large dry well-stoppered container and well shaken. Samples, in duplicate, were removed at different times, in order to obtain powders of suitable moisture contents. The duplicates were placed in dry glass-stoppered bottles, one kept at room temperature and the other at 30° C.

Periodically the solubility and moisture content of the stored powders were determined. The former results are plotted on Graphs II to VII, and the maximum and minimum figures for the latter are indicated below the corresponding graphs.

THE EFFECT OF MOISTURE.—Graph V, showing the change of solubility of full-cream milk powders of different moisture contents at 30° C., demonstrates that powders containing moistures in excess of, or approximating to, the critical moisture content become insoluble at a greater rate than do those which have not reached this point. This is also borne out by Graphs II (Curves C to G) and VI, for separated powder, assuming that the critical moisture content may be calculated for a separated powder from the solids-not-fat content. This assumption appears to be justifiable, since the ratio of critical moisture content to solids-not-fat has been shown to be constant with powders varying from 20 to 32 per cent. fat.<sup>1\*</sup>

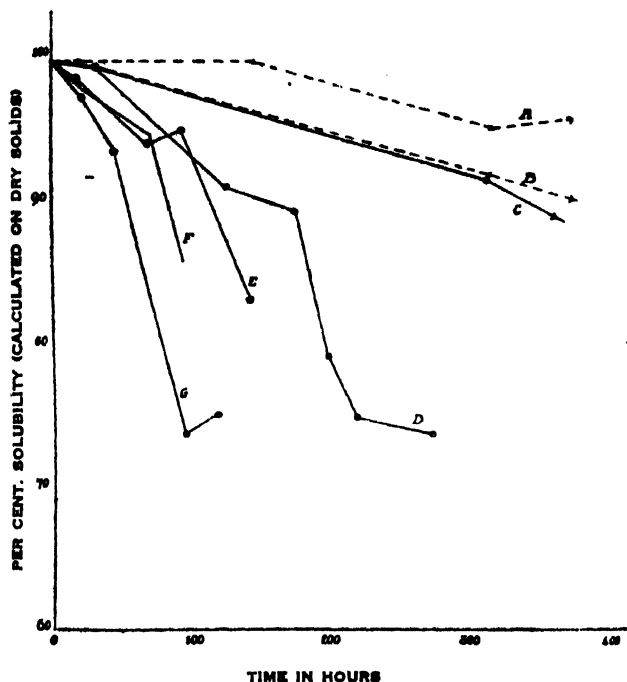
THE EFFECT OF TEMPERATURE.—It will be seen, on reference to Graph II, that, comparing powders of similar moisture content, the change of solubility at room temperature (Curves A and B) is much slower than that at 30° C. (Curves D, E, F and G).

If the solubilities of full-cream milk powders are corrected for fat, in order to bring them into line with the separated milk powders, the difference between the rates of change at the two temperatures, as demonstrated by Graphs III and IV, is seen to be not nearly so great as for corresponding separated powders.

\* It should be noted that, in the above-mentioned paper, reference to the tables of results shows that there is a printing error in the ordinates (percentage of moisture) of Fig. I.



**THE EFFECT OF CORRECTION FOR FAT.**—The general shape of the curves is not materially affected by the refinements in the method of determining solubility (correcting for fat in the insoluble residue, etc.), as mentioned under Section III, as the effects are approximately of equal value and of opposite sign, but certain irregularities, caused by variations in the amount of fat carried down, are certainly smoothed out. This is illustrated by Graph VII.



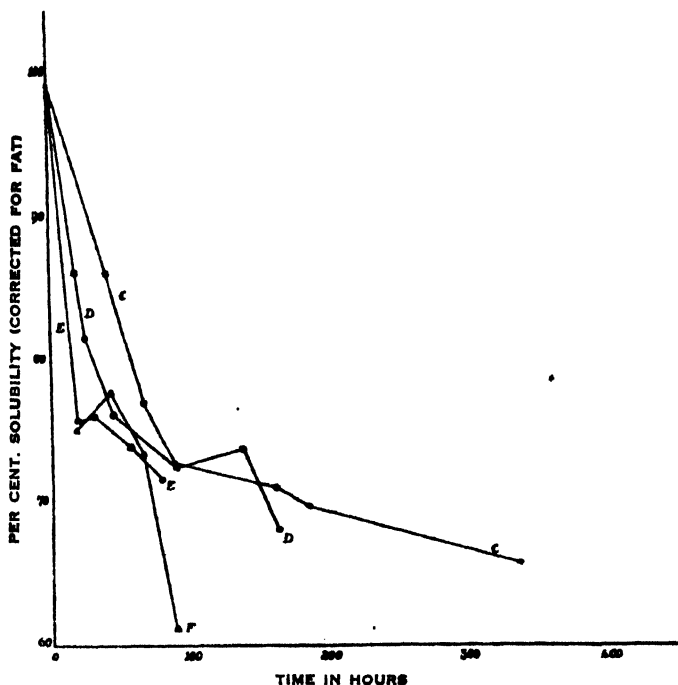
GRAPH II.

The change in solubility of separated milk powders of different moisture contents when kept at room temperature and at 30° C.

	Temperature.	Per cent. moisture.	
		Calculated on dry solids.	Calculated on dry solids-not-fat.
Curve A.	Room temp.	11.1-12.7	11.2-12.9
" B.	" "	10.5-12.5	10.6-12.6
" C.	30° C.	8.5-10.4	8.6-10.5
" D.	30° C.	9.5-13.2	9.7-13.4
" E.	30° C.	11.7-13.2	11.9-13.4
" F.	30° C.	11.5-12.8	11.7-12.9
" G.	30° C.	10.8-12.5	10.9-12.7
Critical moisture content (calculated)		12.3-12.5	12.4-12.7

The general shape of the curves (Graphs V and VII), before correction for fats, is of decided interest; it will be observed that, after a minimum solubility,

there is a decided upward tendency, which tendency is removed when the fat correction has been applied (Graph VII). We consider that the action of moisture is to cause a disruption of the particle of powder, and have previously suggested that this is caused by the crystallisation of the lactose. In the early stages of the action the disruption is not complete, and, consequently, on mixing with water, the precipitated casein brings down entrained a proportion of the fat. In the later stages, disruption having proceeded further, this entrainment of fat is not so appreciable, with a consequent raising of the apparent solubility.



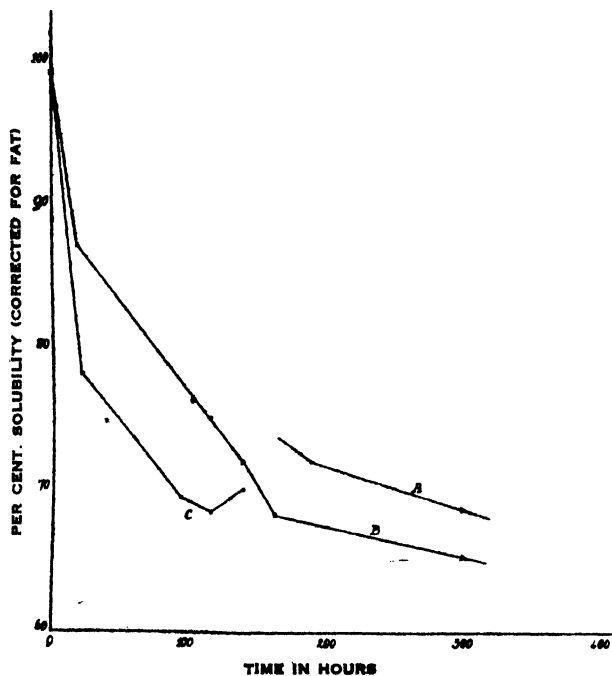
GRAPH III.

Solubility curves C, D, E and F (corrected for fat) correspond with the similarly lettered curves of Graph V.

It must be emphasised that these results apply to spray-process powders.\* As mentioned previously, other workers have studied the problem in part. Of these, we would briefly refer to the work of Fouassier<sup>13</sup> and of Supplee and Bellis.<sup>7</sup> That of the former is of qualitative and not quantitative character, and is somewhat suggestive, in that it puts forward a definite theory regarding what the author calls the "insolubilisation" of milk powder. He makes, however, one statement—based on extremely slender published evidence—that roller process milk powder is fully soluble when tested immediately after manufacture, and

\* In all the work reported herein, milk powders made on the "Milkal" process have been used.

attributes the low solubility of the commercial product to the effect of the 6 per cent. of moisture normally left in the manufactured product. Supplee and Bellis make a similar statement. In their paper, which was designed with special reference to storage problems, the changes investigated are studied over periods up to a year. They found that the effect of the absorption of moisture and consequent development of insolubility was the same, both for powders made on the Just Double-Roller process and the Spray process.



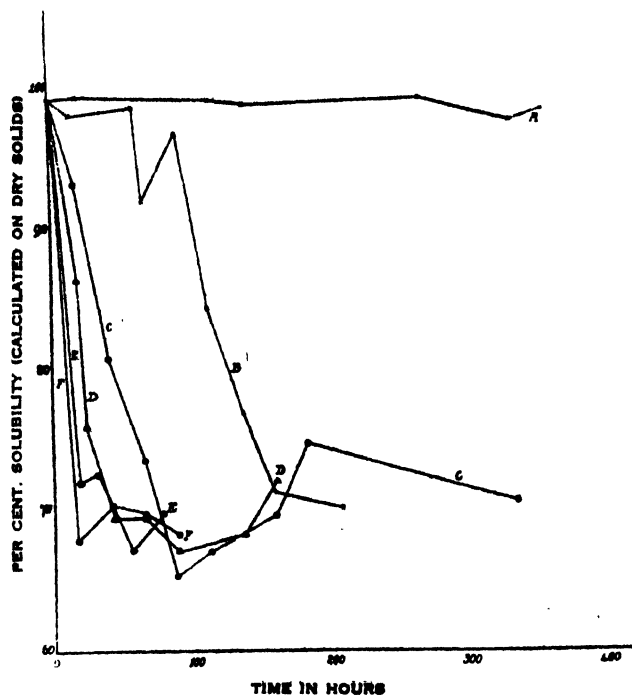
GRAPH IV.

The change in solubility of full-cream milk powder when kept at room temperature.

				Per cent. moisture.	
				Calculated on dry solids.	Calculated on dry solids-not-fat.
Curve A.	..	..	..	9.1-10.5	13.0-15.0
" B.	..	..	..	9.7-10.8	13.9-15.5
" C.	..	..	..	9.5-10.8	13.6-15.4
Critical moisture content	..			8.7- 8.8	12.4-12.7
(calculated)					

We have not had an opportunity to test these statements, but it is probable, as suggested by Fouassier, presuming that Roller-Process powder is completely soluble immediately it is prepared, that the comparatively large amount of moisture which milk powder made on this process usually contains is the cause of the low solubility, so far as the casein is concerned.

Supplee and Bellis tested only one sample of each type of powder, but it is probably of importance that the Just powder used had a moisture content of 1.95 per cent., which is of the order of that found in full-cream spray-process powder.



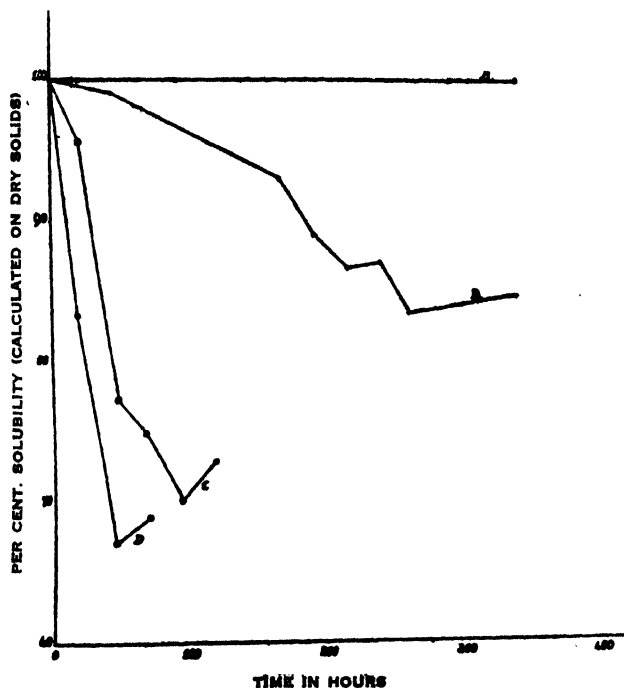
GRAPH V.

The change in solubility of full-cream milk powders of different moisture contents when kept at 30°C.

	Per cent. moisture.	
	Calculated on dry solids.	Calculated on dry solids-not-fat.
Curve A. . . . .	4.2- 5.6	5.9- 8.1
" B. . . . .	4.9- 7.9	7.1- 9.6
" C. . . . .	8.3- 9.7	11.8-13.8
" D. . . . .	9.2-11.1	13.1-15.8
" E. . . . .	9.9-11.5	14.2-16.4
" F. . . . .	10.2-10.9	14.6-15.6
Critical moisture content (calculated)	8.7- 8.8	12.4-12.7

V. THE DISTRIBUTION OF THE DIFFERENT PROTEIN CONSTITUENTS BETWEEN THE SOLUBLE AND THE INSOLUBLE FRACTIONS.—The difficulty experienced by us in determining the solubility of "low solubility" powders, owing to the excessive frothing, suggested that the proteins left soluble after absorption of moisture were albumin and globulin. Moreover, a preliminary examination showed that the

insoluble fraction was largely protein, and knowledge of the distribution of protein between the soluble and insoluble portions appeared desirable. A working hypothesis seemed to be that the absorption of water had little or no effect on the albumin and globulin, but that it caused "denaturation" of the caseinogen, or, perhaps more simply, a splitting of the caseinogen molecule, thus producing the difficultly soluble casein.



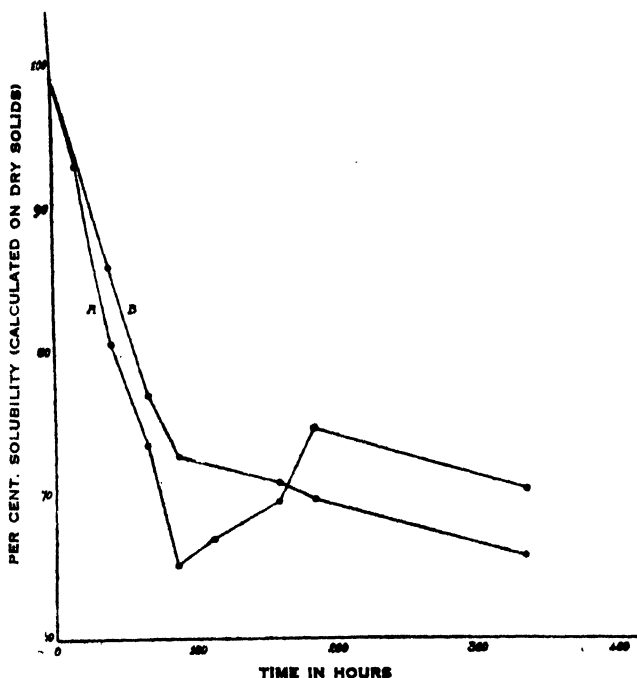
GRAPH VI.

The change in solubility of separated milk powder of different moisture contents when kept at 30° C.

				Per cent. moisture.	
				Calculated on dry solids.	Calculated on dry solids-not-fat.
Curve A.	..	..	..	5.9- 8.7	6.0- 8.8
" B.	..	..	..	10.3-11.9	10.4-12.0
" C.	..	..	..	12.0-14.6	12.2-14.8
" D.	..	..	..	14.1-15.1	14.2-15.3
Critical moisture content (calculated)				12.3-12.5	12.4-12.7

A critical survey of the literature demonstrates the large number of methods which have been employed for the fractionation of the proteins of milk. Some of these methods (*e.g.* those of Howe<sup>14</sup> or of Thorell<sup>15</sup>) divide the proteins into a comparatively large number of fractions; such a fractionation did not appear to

be necessary for the purposes of the work as reported in this paper, and, consequently, attention was directed to the work of Moir,<sup>16</sup> who has lately studied the separation of milk proteins for analytical purposes, and we have adopted, in general, his scheme of separation. His actual technique for the determination of casein was not used, because it was realised that a micro-method would be very much more simple and rapid. The method employed by us, as described below, is based upon the determination of the nitrogen content of the solutions before and after precipitation of the different protein fractions.



GRAPH VII.

Two solubility curves of full-cream milk powder kept at 30° C. Curve A represents the solubility calculated on dry solids, and Curve B represents the same results corrected for fat.

These curves correspond with Curve C in Graphs III and V.

**OUTLINE OF METHOD OF ANALYSIS.**—A solubility determination was carried out in the manner described in Section III, except that the water was weighed into the centrifuge tube. The supernatant liquid, after centrifuging and removal of the fat layer in the case of full-cream powder, was then subjected to differential protein precipitation.

The apparatus used was a centrifugal device illustrated in Fig. 1. It consists of a Jena glass Buchner funnel\* passing through a rubber bung into a small bottle.

\* Funnel No. 3 G4, made by Schott & Gen., Jena, Germany.

The whole is of such dimensions that it may be placed in the bucket of a centrifuge. A solution containing suspended matter may thus be easily filtered, even if it be of the type which readily froths.

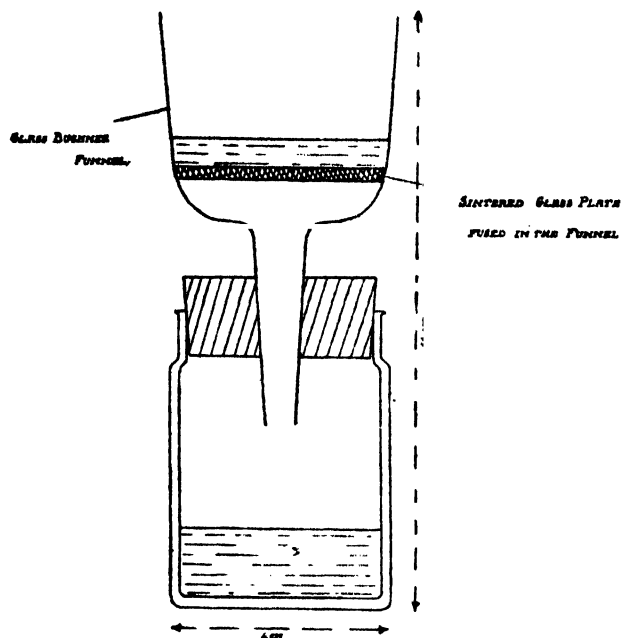


FIG 1.

**PRECIPITATION OF CASEIN.**—The liquid containing the soluble fractions was first filtered by means of the centrifuge device. The bottle portion was removed and fitted with a dropping pipette. Weighed samples of the filtered liquid were removed for nitrogen determinations in triplicate by the micro-Kjeldahl method, and a further portion of 5 c.c. (accurately measured) was taken for the protein precipitation. This volume of 5 c.c. was weighed into a similar centrifuge bottle, 25 c.c. of water (40 to 42° C.) and 0.75 c.c. 10 per cent. acetic acid solution added, stirred with a rod of known weight and allowed to stand for 20 minutes. Sodium acetate solution (2.25 c.c. of 0.25 *N*) was added, and the solution was gently stirred, left for 1 hour, and then weighed. The rod was removed, the tube centrifuged for 3 minutes to separate the precipitated casein, and the supernatant liquid was removed and filtered as described above.

**PRECIPITATION OF ALBUMIN AND GLOBULIN.**—The filtrate represented the soluble fraction, less the casein. Micro-Kjeldahl determinations were again carried out in triplicate, and the whole of the solution then remaining, after weighing, was treated (at 60 to 65° C.) with 50 per cent. trichloroacetic acid in such quantity that the final concentration of acid was 4 per cent. The liquid was stirred, allowed to stand for 30 minutes (at 60 to 65° C.) and centrifuged, the supernatant liquid

was filtered, and the whole of the filtrate was used for a micro-Kjeldahl determination of the non-protein nitrogen. From determinations of the nitrogen in the original powder and in the various liquids obtained, casein, albumin and globulin, and non-protein nitrogen were calculated.

The scheme of analysis, as outlined, was applied to a number of powders detailed in Table III.

TABLE III.  
DETAILS OF POWDERS USED FOR PROTEIN ANALYSIS.

Type of milk powder.	Sample No.	Conditions of keeping.	Solubility			
			Moisture Per Cent.	Fat. Per Cent.	as determined Per Cent.	corrected for fat. Per Cent.
Separated milk powder	I	—	2.01	1.23	99.84	
	II	Room temp. 30° C.	15.8	1.23	69.9	
	III	—	14.5	1.23	69.1	
Full-cream milk powder	IV	—	1.27	30.18	99.61	
	V	Room temp. 30° C.	12.3	30.18	68.4	64.6
	VI	—	10.3	30.18	68.3	61.2

The percentage moisture, fat, and solubility, are all calculated on the dry solid content of the powder. The results of the protein analysis of the soluble fractions of the milk powders are shown in Tables IV and V; in the former, the figures are calculated on the total solids of the milk powder, but, in order to make comparison between the results for full-cream milk powder and separated milk powder possible, the figures are shown in Table V, calculated as a percentage of the total protein. In all cases we have used the factor  $N \times 6.38$  to calculate protein from the nitrogen content.

TABLE IV.  
RESULTS CALCULATED AS PERCENTAGE OF TOTAL SOLIDS.

Sample No.	Separated milk powder.						Full-cream milk powder.				
	Original.		After treatment.			III.	Original.		After treatment.		
	I.A.	I.B.	II.A.	II.B.	III.		IV.A.	IV.B.	V.A.	V.B.	VI.
Soluble protein .. ..	36.2	36.8	7.4	7.3	7.2		26.4	27.1	3.6	3.4	3.3
Casein (protein pptd. by acetate buffer) .. ..	28.8	29.3	1.8	2.0	1.9		22.6	22.6	1.1	0.8	0.6
Albumin and globulin (protein pptd. by trichloro-acetic acid) .. ..	5.1	4.9	4.0	3.5	3.7		2.2	2.3	1.0	1.8	1.3
Non-precipitated nitrogen (calc. as protein) ..	2.3	2.6	1.6	1.8	1.6		1.6	2.2	1.5	0.8	1.4



TABLE V.

## RESULTS CALCULATED AS PERCENTAGE OF TOTAL PROTEIN.

Sample No.	Separated milk powder.					Full cream milk powder.				
	Original.		After treatment.			Original.		After treatment.		
	I.A.	I.B.	II.A.	II.B.	III.	IV.A.	IV.B.	V.A.	V.B.	VI.
Soluble protein .. ..	95.2	96.9	19.4	19.3	18.9	96.7	99.5	18.1	12.6	12.1
Casein (protein pptd. by acetate buffer) .. ..	75.8	77.1	4.8	5.3	5.0	82.9	83.1	4.0	2.9	2.4
Albumin and globulin (protein pptd. by trichloroacetic acid) .. ..	13.4	12.9	10.4	9.3	9.7	8.0	8.4	3.8	6.6	4.7
Non-precipitated nitrogen (calc. as protein) ..	6.0	6.9	4.2	4.7	4.2	5.8	8.0	5.3	3.1	5.0

In Tables III, IV and V, samples I.A. and I.B. are taken from the original separated milk powder, whilst samples IV.A. and IV.B. are from the original full-cream milk powder. Samples II.A., II.B., V.A., and V.B. are powders after absorption of moisture, kept at room temperature, the first two being separated powders and the last two full-cream milk powders. Finally, Samples III and VI are, respectively, separated and full-cream powders kept at 30° C. subsequent to the absorption of moisture.

It must be realised that we have not succeeded in bringing the solubility down to the point where the whole of the casein is insoluble, although, calculated on the total protein, the casein has descended from 77.1 per cent. of soluble to 4.8 per cent. of soluble substance in the extreme case for separated powder, and from 83.1 per cent. to 2.4 per cent. for full-cream powder. This is not in agreement with the work of Supplee and Bellis,<sup>7</sup> who, in practically every test, found no casein in suspension after treatment. Certain it is that in all cases after treatment the method of precipitation used by us (Supplee and Bellis do not describe their technique) gave a precipitation of casein. The distribution of the proteins before and after the change of solubility is being further investigated by us, but it can be definitely asserted that our results show that, whilst the soluble portion contains only a small proportion of the casein originally present, a relatively large proportion of the albumin and globulin fractions remains soluble.

It would appear that deductions of a similar nature can be drawn from a study of the figures given by Supplee and Bellis in the paper to which reference has already been made. They stored Just Double-Roller process milk powder for varying periods of time up to 369 days, traced the changes in moisture content, and correlated this with determinations of soluble protein and casein in suspension in reconstructed milk made from the various samples. No further differentiation of the proteins was attempted, and, whilst they only comment on the progressive decrease in the amount of casein in suspension, suitable calculations can be made from their results, which show that the protein other than casein does not materially change.

Fouassier<sup>18</sup> also states (although his contention is not supported by any analytical figures) that milk powder can be rendered insoluble by absorption of moisture, and, moreover, indicates that the reaction is accelerated by heat. He puts forward the interesting theory that the action is due to the natural acidity of the fresh milk, which in the dry state cannot bring about the insolubility of the casein, but which, in the presence of a small amount of absorbed moisture, is sufficiently concentrated to cause this effect. The theory requires investigation, but this effect cannot be that of the concentration of the natural acidity only; time must be an important factor, otherwise the insolubility would be produced during the actual drying process when the powder must obviously pass through a stage when the same acid concentration would be reached. Yet, according to Fouassier, freshly prepared roller-process powder is completely soluble.

GENERAL SUMMARY AND CONCLUSIONS.—1. A study has been made of the absorption of water by milk powder, with particular reference to its effect on solubility.

2. The application of a torsion balance method, whereby changes of moisture content of masses of powder of the order of 0.02 to 0.20 grm. can be determined, has revealed that roller-process powder absorbs moisture in a regular manner, but that, in the case of spray-process powder, the action is much more complicated, and that, before equilibrium is reached between the powder and the atmosphere, a maximum moisture content is reached, followed by a subsequent loss.

3. Very definite indications have been found that this peculiar phenomenon is due to the lactose contained in the powder, for spray-dried lactose itself absorbs moisture up to about 10 per cent., and subsequently loses practically the whole of this moisture. Moreover, this action is unique with spray-dried lactose, other forms of lactose showing no such absorption and loss.

4. Methods have been described whereby the course of the absorption has been followed on a large scale and the resultant changes in solubility studied. The difficulty of obtaining a mass of powder of homogeneous moisture content has been indicated, the difficulty increasing with the moisture content; this has been attributed to the peculiarity mentioned in paragraph 2.

5. A technique has been described for the determination of solubility with due reference to the influence of fat.

6. The effect of the absorption of moisture has been shown to be the lowering of the solubility of the milk powder; the rate at which the solubility decreases has been proved to be dependent on at least two factors: moisture content and temperature.

7. In the case of full-cream powder below a moisture content of approximately 6 per cent. the decrease in solubility with time is almost negligible; in the region of the critical moisture content, the change is extremely rapid. In the case of separated milk powder the same findings hold, but the moisture range of no change, of slow change, and of rapid change, is materially higher than for

full-cream powder, owing to the fact that the critical moisture content is proportionate to the solids-not-fat.

8. Temperature has been shown to be an important factor in determining the rate at which solubility is lowered; the lower the temperature, the slower the rate. Hence, the higher the temperature, the more is the difference in solubility between powders of low and of high moisture content accentuated.

9. Above the critical moisture content, time and temperature have no effect on the final solubility, which, calculated on solids-not-fat, is the same in the case of both full-cream and of separated powders.

10. A study has been made of the distribution of casein on the one hand, and of globulin and albumin on the other, in the soluble and in the insoluble fractions of milk powder which has absorbed moisture, and become relatively insoluble. The technique employed has been described; the results show that the insoluble portion is essentially casein, whilst the soluble portion contains almost the whole of the albumin and globulin. These facts have been associated with the "frothing" qualities of milk powders of low solubility.

11. It has been shown that the apparent solubility of full-cream milk powders which have been rendered partly insoluble by absorption of moisture is affected by the amount of fat carried down with the insoluble protein. The amount of fat thus brought down is shown to vary, and may be as much as 40 per cent. of the precipitate. Corrections have been made for this, giving curves which are much more regular.

Our thanks are due to Messrs. J. Lyons & Co., Ltd., in whose laboratories this work was conducted, for permission to publish; also to Misses F. I. Poyser, B.Sc., and D. F. Filmer, B.Sc., who have carried out a large portion of the experimental work, and to Mr. E. B. Hughes, M.Sc., F.I.C., for valuable criticism.

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# Investigations into the Analytical Chemistry of Tantalum, Niobium, and their Mineral Associates.

## XX. The Separation of Tin from Tantalum and Niobium.

By W. R. SCHOELLER, PH.D., AND H. W. WEBB.

(*Work done under the Analytical Investigation Scheme.*)

(*Read at the Meeting, May 6th, 1931.*)

STANNIC oxide is a regular minor constituent of the majority of earth-acid minerals, and hence the mineralogist requires a process for the separation of a small quantity of tin from much earth acid. The converse case—small amounts of earth acid from much tin—arises in the analysis of certain varieties of cassiterite containing tantalic acid (ainalite; *U.S. Geol. Survey Bull.* 509, 1912, p. 20), and of alluvial tin deposits mechanically admixed with tantalite or columbite. The two cases will here be considered, as well as the separation of earth acid, tin oxide, and silica.

**I. SEPARATION OF SMALL AMOUNTS OF TIN FROM MUCH EARTH ACID.—**  
*Older Methods.*—Those of Berzelius and Rose have already been noticed and adversely criticised in Section VIII (ANALYST, 1927, 52, 507, 509), hence it is unnecessary to return to the subject. We will confine ourselves to adding yet another quotation from the literature, indicating that the older methods did not fully accomplish their object: "Sample D [tantalite] contains quite large amounts of tin, difficult to remove from the niobium and tantalum, as experience has shown. This tin accumulates in the niobium portion in the crystallisation [Marignac's double fluoride] method, thus causing high results for niobium." (Metzger and Taylor, *School of Mines Quarterly*, 1909, 30, 332.)

*Recent Methods.*—Three processes will be discussed: the first two—Simpson's and Giles's—utilise an alkaline, the third—Schoeller and Powell's—an acid flux.

**A. SIMPSON'S PROCESS.**—Simpson has described a process for the determination of tantalum in tantalite (*West Austral. Geol. Survey Bull.* 23, 1906, p. 72; *Chem. News*, 1909, 99, 243), which is an improvement over the old pyrosulphate hydrolysis method (XII, ANALYST, 1928, 53, 467). The finely-powdered mineral is fused with potassium hydroxide in a nickel crucible, the product disintegrated with dilute hydrochloric acid, and the earth acids precipitated by boiling with a definite excess of the acid, when they "remain insoluble except in traces." The precipitate consists of the "hydrates of Ta, Nb (and W?), with all Sb, and at times traces of  $Mn_2O_3$  and  $SnO_2$ . The filtrate contains all Sn, Fe, Mn, Ca, Mg, Cu, Ni, and Ti as chlorides." According to these indications the method provides a separation of tin, but not of antimony, from the earth acids; Simpson conducts

the determination of the tin in the filtrate from the hydrolysis precipitate by saturating the liquid with hydrogen sulphide and igniting the precipitate to  $\text{SnO}_2$ .

We made an experimental investigation of the completeness of the separation. After some preliminary experiments (which proved that a lower acid concentration than that prescribed by Simpson caused extensive co-precipitation of tin with the earth acids), the following four separations were made in accordance with the original directions. The filtrate from the earth-acid precipitate, *HP*, obtained by hydrochloric acid, was saturated with hydrogen sulphide, and the precipitate collected and cautiously ignited to stannic oxide (*SnP*) by known methods:

Exp.	Grms. taken.		<i>HP</i> .	Apparent		$\text{SnO}_2$ error.	$\text{SnO}_2$ in <i>HP</i> . (a)
	$M_2O_5$ .	$\text{SnO}_2$ .		$M_2O_5$ error.	<i>SnP</i> .		
1	0.2053	0.0203	0.2038	-0.0015	0.0190	-0.0013	
2	0.2012	0.0220	0.1998	-0.0014	0.0211	-0.0009	
3	0.2002	0.0232	0.1985	-0.0017	0.0239	+0.0007(b)	0.0017
4	0.2012	0.0224	0.1994	-0.0018	0.0213	-0.0011	0.0014

(a) *HP* was tested for Sn by method C: in Exps. 1 and 2 Sn was detected, but not actually determined.

(b) The combined *SnP* from Exps. 3 and 4 was tested for  $M_2O_5$  by method C: a characteristic tannin precipitate (0.0016 gm.) was obtained, no doubt chiefly derived from Exp. 3 with a positive tin error.

The above table shows that, with the exception of (b), all the observed errors are negative. As to the incomplete recovery of the tin, the last column explains that the small missing fraction was found in *HP*; hence the separation of tin from tantalum and niobium by Simpson's process was not quite quantitative. This is not surprising when the adsorptive capacity of earth-acid precipitates is borne in mind. That they may contain "traces" of tin is admitted by Simpson, while Mellor (*A Treatise on Quantitative Inorganic Analysis*, London, 1913, p. 419) states that they contain "some of the tin." At the same time, it appears that Simpson's process furnishes at least as effective a means for the separation as any so far published.

Although *HP* has been shown to be slightly contaminated with tin, the apparent earth-acid errors are still negative. Hence the actual error is greater still, *i.e.* -0.0025 to -0.003 gm. Now Simpson states that the earth acids "remain insoluble except in traces." We considered the subject of hydrochloric acid hydrolysis sufficiently interesting to check this statement by working with the pure pentoxides without adding stannic oxide. Three tests were accordingly made as before; the filtrates from *HP*, however, were worked up for the missing fraction. This was done by approximate neutralisation, boiling, and addition of ammonium acetate and tannin, which gave the precipitates *TP*. These were ignited and purified as under XVII (ANALYST, 1929, 54, 710):

Exp.	Grms. taken.	<i>HP</i> .	<i>TP</i> .
5	$\text{Ta}_2\text{O}_5$ 0.2034	0.1996	0.0034
6	$\text{Nb}_2\text{O}_5$ 0.2072	0.2027	0.0032
7	do. 0.2026	0.1981	0.0032

The results confirm the preceding ones, proving Simpson's process to result in a loss of about 0.003 to 0.004 grm. of pentoxide. Whilst such error may perhaps be tolerated in technical work, it would have to be rectified in an accurate mineral analysis.

In conclusion, Simpson's process gives low results for both earth acids and tin: the former, because they are not quite quantitatively precipitated; the latter, because a small amount of tin remains in the earth-acid precipitate.

*Identical Effect of Hydrochloric Acid Hydrolysis and of Tartaric Hydrolysis.*—Before leaving the subject, we would draw a useful parallel between hydrochloric (Simpson's) hydrolysis and tartaric hydrolysis (XVI, ANALYST, 1929, 54, 704). The latter consists in boiling a tartrate solution of the earth acids with a large excess of hydrochloric (nitric) acid, and it results, like the former, in a shortage of a few mgrms. of pentoxide. In other words, when the mineral acid has broken up the soluble tartaric earth-acid complex, the effect of the tartaric acid upon the earth acids is eliminated, and the reaction reverts to simple hydrochloric hydrolysis, an unfinished reaction resulting in slightly incomplete precipitation. This interpretation enables us to rectify the view (expressed in IX, ANALYST, 1927, 52, 634) that the incomplete precipitation in tartaric hydrolysis is "possibly due to the presence of sulphate ion (from the flux) or interference of titania"; since neither was present in Exps. 5 to 7, they can hardly be made responsible for the negative errors observed in Section IX.

**B. GILES'S METHOD** (*Chem. News*, 1909, 99, 1).—The fine powder is fused at high temperature with potassium carbonate, and the melt digested with citric acid solution. The insoluble residue from the operation (if any) is once more submitted to fusion and citric acid extraction. The tin is precipitated from the combined acid extracts as sulphide; the earth acids are recovered from the filtrate after destruction of the citric acid.

One disadvantage of the above scheme (which Giles does not appear to have tested quantitatively) is, that stannic oxide is imperfectly attacked by the flux, a fact recorded in the literature and confirmed by our own tests: Giles has to provide for a repetition of the treatment. At the high temperature required, the platinum crucible is slightly attacked, with the result that a little platinum is dissolved and precipitated as sulphide with the tin. This constitutes another disadvantage, "as the separation of tin from platinum is perhaps as difficult (if not more so) as from any of the constituents" (Thornton, *Titanium*, New York, 1927, 168). As for the application of Giles's scheme in mineral analysis, the only mention in the literature is a discouraging account by Wells (*Amer. J. Sci.*, 1911, 31, 436), who used it in an analysis of strüverite. After three fusions, a very low result was obtained, namely, 0.7 per cent. of  $\text{SnO}_2$ , as against 1.7 per cent. by a different method.

It will be seen that the available information is unfavourable towards Giles's method. Now the whole of our experience has led us to abandon alkaline fluxes for the decomposition of earth-acid minerals. Schoeller and Powell (X, ANALYST,

1928, 53, 263) have already shown the disadvantage of alkaline flux in rendering silica soluble and thus complicating its separation from the earth acids. It must be obvious that the introduction of yet another constituent—stannate—will make matters worse, especially as the fairly refractory stannic oxide may still partly remain in the residue from the fusion, while platinum derived from the crucible will contaminate the soluble fraction.

After due consideration of these difficulties, we decided that a quantitative investigation of Giles's method would serve no useful purpose, the procedure not appearing to be suitable for the analysis of earth-acid minerals.

C. SCHOELLER AND POWELL'S METHOD.—This method, outlined in Section I (ANALYST, 1922, 47, 93), consists in bisulphate fusion of the mineral, lixiviation with tartaric acid, and treatment of the solution with hydrogen sulphide. Most of the stannic oxide remains insoluble, whilst the earth acids dissolve. The small amount of dissolved tin is precipitated as sulphide; residue and precipitate are collected, ignited, and weighed as  $\text{SnO}_2$ . We tested the proposed method by working with mixtures of the pure oxides and applying the following procedure:

THE SEPARATION.—The mixed oxides are fused with bisulphate, and the product leached with tartaric acid or ammonium tartrate solution as prescribed in XIX, A, B, (ANALYST, 1931, 56, 305). The treatment results in a solution containing the bulk of the earth acids and a few mgrms. of tin, and an insoluble fraction consisting of stannic oxide with a variable but small amount of undissolved earth acid.

The crucible contents having been quantitatively transferred to a 250 c.c. beaker, the unfiltered solution is acidified with an excess of 5 c.c. of sulphuric acid (1:1), and treated with 2 to 3 c.c. of one per cent. mercuric chloride solution and a current of hydrogen sulphide at about  $50^\circ\text{C}$ . When the precipitate,  $P^1$ , has coagulated, a little filter pulp is stirred in to entangle finely-divided tin oxide, and the precipitate collected on a 9 cm. No. 40 Whatman filter and washed with hydrogen sulphide water containing a little sulphuric acid. The filtrate,  $F^1$ , is concentrated by evaporation in a 600 c.c. beaker, while  $P^1$  is gently ignited in the original silica crucible.

Re-treatment of  $P^1$ .—The ignited precipitate contains the whole of the tin, and, normally, a small residual admixture of earth acid. Hence it should always be re-treated in precisely the same manner as the original oxides. From the appearance of the sulphide precipitate we judge that the attack on the tin oxide is always more vigorous in the second fusion. The re-treatment furnishes a precipitate  $P^2$  and a filtrate  $F^2$ . The former is ignited in a tared porcelain crucible and weighed as  $\text{SnO}_2$ ; the latter is concentrated by evaporation, and reserved.

Testing  $P^2$  for Purity.—In absence of silica, the weight of  $P^2$  represents the amount of stannic oxide in the mixed oxides. A negative error may be due to finely-divided tin oxide running through the filter, but this should be adequately prevented by the addition of mercuric salt and pulp, and re-filtration if necessary.

In accurate work, the purity of  $P^2$  should always be tested, preferably by the following process. The weighed precipitate is transferred to a porcelain boat, which is heated to a moderate red heat in a combustion tube in a stream of hydrogen. The oxide first darkens considerably, then becomes gradually converted into pale-grey metallic tin. After cooling in hydrogen, this is dissolved in warm hydrochloric acid, with or without bromine, the solution filtered, and any insoluble residue washed, ignited, and weighed; it may be tested for earth acid. The tin in the filtrate may be determined gravimetrically by precipitation with ammonia or hydrogen sulphide, or volumetrically by the iodine process.

*Recovery of the Earth Acids.*—The filtrate  $F^1$ , concentrated to about 150 c.c., is boiled with 25 c.c. of strong hydrochloric acid, yielding the bulk of the earth acids as the hydrolysis precipitate  $HP$ . The filtrate from  $HP$  is united with the concentrated filtrate  $F^2$  and treated for the recovery of the balance of the earth acids as the tannin precipitate  $TP$ . These operations are fully described in XIX, C, E, F (*loc. cit.*).

*Separation in Presence of other Heavy Metals.*—If other metals of the hydrogen sulphide group are present, the tin will be obtained in two fractions, the above process being modified as follows: the residue  $R^1$  from the first fusion and lixiviation is collected, washed, and ignited, and once more submitted to the same cycle of operations. The final residue  $R^2$  is weighed as  $SnO_2$ ; the balance of the tin is in the combined filtrates from  $R^1$  and  $R^2$ , together with the other members of the hydrogen sulphide group. The separation of the earth acids from that group will form the subject of a subsequent section (XXII).

RESULTS OF TEST SEPARATIONS.—The subjoined Table contains the results of experiments with tantalum, niobium, and mixed pentoxides (see column 1). In Exps. 8 to 12, the composition of the oxide mixture was not divulged to the operator till he had carried out the separation. The weight of  $P^1$  was ascertained because it records the amount of earth acid left in the residue from the first operation. In Exps. 13 and 14, the tin was recovered in two separate fractions as explained in the preceding paragraph:  $R^2$  represents the insoluble stannic oxide, while  $SP$  is the ignited sulphide precipitate obtained in the combined filtrates from  $R^1$  and  $R^2$ .

Exp.	Grms. taken.		$P^1$ .	$P^2$ .	$SnO_2$ error.	$HP$ .	$TP$ .	$M_2O_5$ error.
	$M_2O_5$ .	$SnO_2$ .						
Ta 8	0.1232	0.0543	0.0564	0.0543	0.0000	0.1142	0.0085	-0.0005
" 9	0.2534	0.0204	0.0440	0.0204	0.0000	0.2216	0.0320	+0.0002
" 10	0.1558	0.0642	0.0708	0.0633	-0.0009(a)	0.1413	0.0152	+0.0007(a)
Nb 11	0.2013	0.0202	0.0230	0.0211	+0.0009	0.1700	0.0313	0.0000
" 12	0.1555	0.0499	0.0542	0.0498	-0.0001	0.1106	0.0456	+0.0007
			$R^2$	$SP$		(a) See text below.		
EA 13	0.2083	0.0435	0.0350	0.0083	-0.0002			
" 14	0.2130	0.0227	0.0131	0.0099	+0.0003			

The errors observed in Exp. 10 made us suspect that a minute amount of tin had found its way into the earth-acid filtrates, either as finely-divided oxide or by escaping precipitation as sulphide. We proceeded to search for the missing



tin fraction by the method under discussion, as a test of its efficiency for very small quantities. Accordingly, the recovered ( $HP+TP$ ) was again submitted to the process, the tartrate solution giving a faint sulphide precipitate. This was ignited, and reduced in hydrogen; the residue gave a definite tin reaction.

We submit that the results of the test analyses are satisfactory, proving the process in its present form to furnish a quantitative separation within the limits of experimental error. We apply it in actual practice in the analysis of tantalite.

II. SEPARATION OF SMALL AMOUNTS OF EARTH ACID FROM MUCH TIN.—Of the various processes for converting stannic oxide into a soluble compound, reduction to the metal by ignition in hydrogen is undoubtedly the most elegant; it is especially valuable for the decomposition of substances in which tin oxide preponderates, the analysis of which involves the working up of larger quantities so that minor constituents may be determined. The alternative to reduction would be fusion with an alkaline flux, a mode of working beset with difficulties.

*Procedure.*—The reduction is carried out in a porcelain boat placed in a combustion tube; the boat remains unattacked, hence there is no contamination. We prefer a boat to a Rose crucible, as the progress of the reduction can be watched and the operation is under perfect control. The temperature is regulated to a moderate red heat, and the ignition is interrupted when the whole of the oxide, after transient darkening, has become converted into minute, grey metallic globules. The boat is left to cool under hydrogen, the contents transferred to a 250 c.c. beaker and dissolved in hydrochloric acid and bromine; the liquid is evaporated to dryness on a hot plate and the treatment repeated, when the tin will be eliminated by volatilisation. The residue is digested with dilute hydrochloric acid, and a little filter pulp added; the insoluble matter is collected, washed, ignited in a silica crucible, and fused with bisulphate. The product is dissolved in tartaric acid, the acidified liquid treated with a few drops of mercuric chloride solution and hydrogen sulphide, and the precipitate filtered off. The filtrate is boiled, neutralised with ammonia, treated with ammonium acetate and chloride, and precipitated with tannin as usual: the precipitate,  $TP$ , is ignited to  $M_2O_5$ .

*Exp. 15.*—We applied the above procedure to an intimate mixture of 3.0 grms. of pure tin oxide and 0.0038 grm. of mixed pentoxide (weight unknown to operator). Residue from hydrogen reduction and acid attack: 0.0062; final  $TP$ , 0.0039 grm.; error, +0.0001 grm.

III. SEPARATION OF EARTH ACID, TIN OXIDE, AND SILICA.—The following data on the separation of these three constituents in any proportions are based on the conclusions recorded in this paper and Section X (*loc. cit.*). The separation is based on the solubility of the earth acids in tartaric acid after bisulphate fusion, the reducibility of tin oxide by hydrogen, and the volatilisation of silica by hydrofluoric acid. The order in which these reactions are applied should be adapted to the composition of the material under treatment. We may distinguish three cases:

(1) *Earth Acids Preponderate.*—The substance is fused with bisulphate and

the product extracted with tartaric acid (double treatment). Residue: tin oxide and silica. Filtrate: earth acids and small soluble tin fraction.

(2) *Tin Oxide Preponderates*.—The fine powder is heated in hydrogen, then extracted with hydrochloric acid. Solution: tin; residue: earth acids and silica.

(3) *Silica Preponderates*.—The weighed substance is evaporated with hydrofluoric and sulphuric acids. Residue: earth acids and tin oxide. In the case of silicates, the treatment must be preceded by the customary carbonate fusion and evaporation with nitric acid (not hydrochloric acid, stannic chloride being volatile). The insoluble residue is collected, washed, ignited, and weighed, and the silica volatilised.

*Exp. 16*.—A Nigerian tin ore (72.5 per cent. Sn) was tested for earth acids. The fine powder (5 grms.) was reduced in hydrogen and extracted with hydrochloric acid, the insoluble residue being re-treated. The residue from the second extraction was treated with hydrofluoric and sulphuric acids, then fused with bisulphate, and the mass dissolved in tartaric acid (a). The iron was precipitated with ammonia and ammonium sulphide, the filtrate acidified with acetic acid and boiled with tannin. The ignited *TP* was again brought into tartrate solution, which was boiled with excess of hydrochloric acid: the characteristic white *HP* was ignited, weighed (0.1236 gm.), and tested for titania (0.002 gm.). As a short cut (provided the  $M_2O_5$  content were not too low), solution (a) could be submitted to tartaric hydrolysis.

**SUMMARY**.—The following two cases are considered: (1) Separation of small amounts of tin from much earth acid. Simpson's process (fusion with potassium hydroxide and precipitation of the earth acids with hydrochloric acid) was found to give slightly low results for both tin and earth acid. Giles's process (fusion with potassium carbonate, solution in citric acid, and precipitation of tin as sulphide) is criticised, being shown not to be suitable for earth-acid minerals. Schoeller and Powell's method (fusion with bisulphate, solution in tartaric acid, treatment with hydrogen sulphide, collection of insoluble residue and sulphide precipitate) was investigated and found to be serviceable. (2) Separation of small amounts of earth acid from much tin. The separation is based on the reduction of the tin oxide by hydrogen. Finally, the separation of earth acid, tin oxide, and silica is discussed.

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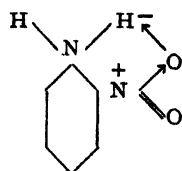
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## The Interaction of Amines and Phenols with Arylsulphonyl Chlorides.

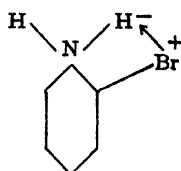
By F. BELL, Ph.D., F.I.C.

OWING to the increasing use of *p*-toluenesulphonyl chloride and *m*-nitrobenzenesulphonyl chloride as reagents for the characterisation of amines and phenols, it appears of value to present a summary of the principal side reactions which may occur.

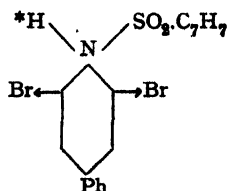
Treatment of an amine with the theoretical amount of the sulphonyl chloride in the presence of a tertiary base, such as pyridine or diethylaniline, usually furnishes the pure sulphonanilide in almost quantitative yield. Two types of divergence from this general behaviour have been noticed (Bell, *J. Chem. Soc.*, 1929, 2787; 1930, 1072). First, polynitroamines, *e.g.* 2:4-dinitroaniline and 3:5-dinitro-2-aminodiphenyl, can be recovered unchanged from this process. In a second class are those amines which, with one molecule of the chloride, give either a mixture of sulphonanilide and disulphonanilide or disulphonanilide alone. Both *o*-nitroaniline with *m*-nitrobenzenesulphonyl chloride and 1:8-dinitro- $\beta$ -naphthylamine with *p*-toluenesulphonyl chloride yield a mixture of mono- and disulphonanilides, whilst 3:5-dibromo- and 3:5:4'-tribromo-4-aminodiphenyls with *p*-toluenesulphonyl chloride and 2-nitro-*p*-toluidine and 6-nitro-*o*-toluidine with *m*-nitrobenzenesulphonyl chloride give only the disulphonanilides. The yield of disulphonanilides is not quantitative even if two molecules of the sulphonyl chloride are employed, so that the velocity of disulphonanilide formation must be high compared with that of monosulphonanilide formation. It has been suggested (Bell, *J. Chem. Soc.*, 1931, 2345) that these peculiarities are associated with the presence in the amine of a chelate ring (I and II). If the ring is of high stability, sulphonanilide formation is inhibited; with diminishing stability the velocity of reaction correspondingly rises. The velocity of disulphonanilide formation is determined by the tendency of the imino hydrogen atom (III\*) to ionise. This,



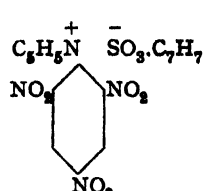
(I)



(II)



(III)

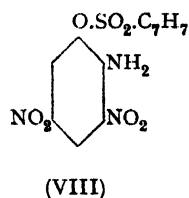
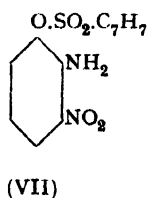
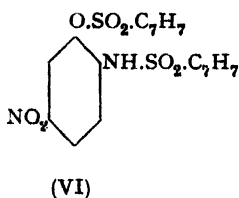
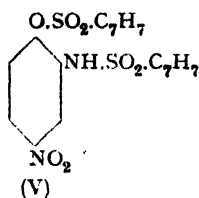


(IV)

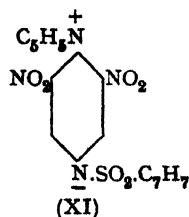
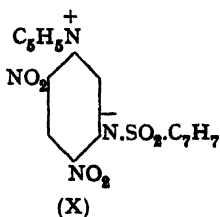
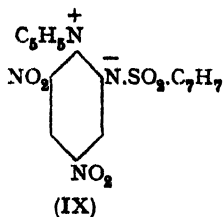
in turn, depends on the presence of electron-absorbing atoms or groups, *e.g.* Cl, Br, I, NO<sub>2</sub>, in the nucleus. The general conclusion can be drawn that arylsulphonyl chlorides are ill adapted to the rapid characterisation of *o*-nitro and *o*-halogenated amines.

All phenols react readily with arylsulphonyl chlorides in pyridine, and, normally, the pure arylsulphonate is the only product. With polynitrophenols, however, other compounds may be formed. If the reaction is conducted in the cold, a pyridinium salt is produced, picric acid, for example, giving picrylpyridinium-*p*-toluenesulphonate (IV) (Bell, *J. Chem. Soc.*, 1931, 609). If the phenol and sulphonyl chloride are heated together, the hydroxyl group becomes replaced by chlorine; under these conditions picric acid gives picryl chloride (Ullmann and Bruck, *Ber.*, 1908, 41, 1870). Consequently, by interaction of a nitrophenol and a sulphonyl chloride there may be produced a mixture of sulphonate, pyridinium salt and chlorohydrocarbon (see, *e.g.*, Borsche and Feske, *Ber.*, 1926, 59, 685). Halogenated phenols behave normally, since Sané and Joshi (*J. Chem. Soc.*, 1924, 125, 2481) record no difficulty in preparing the *p*-toluenesulphonyl derivatives of 2:4:6-tribromophenol, 2:4-dibromo-6-nitrophenol and 2:6-dibromo-4-nitrophenol. On the other hand, 2:4-dinitro-6-bromophenol and 2:4-dinitro-5-chlorophenol readily gave the corresponding chlorohydrocarbons. Arylsulphonyl chlorides are, therefore, of little value for the characterisation of phenols containing two or more nitro groups.

With aminophenols the straightforward formation of an O:N-diarylsulphonyl derivative is liable to complications if nitro groups are present in the nucleus. 4- and 5-Nitro-2-aminophenols react normally (V and VI), but 3- and 3:5-dinitro-2-aminophenols can be converted only into the mono-*p*-toluenesulphonyl derivatives (VII and VIII). When the nitro groups are in *o-p* positions to the hydroxyl



group, as in picramic acid, 2:4-dinitro-5-aminophenol and isopicramic acid, there is produced an inner salt, an anhydro-*p*-toluenesulphonamidopyridinium hydroxide (IX, X and XI). The most certain method of characterisation of a nitroamino-phenol appears to be by solution in acetic anhydride, precipitation with water,



solution of the product in dilute sodium hydroxide, and reprecipitation by acid, when the pure acetamidophenol is obtained.

**SUMMARY.**—Although *p*-toluenesulphonyl chloride and *m*-nitrobenzenesulphonyl chloride, as a rule, give sharply melting, easily crystallised sulphonanilides and arylsulphonates with amines and phenols, respectively, their use is not to be recommended with halogenated amines, polynitroamines and polynitrophenols. Nitroaminophenols appear to be best characterised by preparation of the acetamidophenols.

BATTERSEA POLYTECHNIC, S.W.11.

## The Direct Determination of Nitrogen in Gases.

By H. R. AMBLER, B.Sc., F.I.C.

(*Read at the Meeting, November 4th, 1931.*)

**INTRODUCTION AND SYNOPSIS.**—The general problem of the determination of nitrogen in gases (or in bodies that can be converted into gases) resolves itself into the removal of all such gases as can be removed chemically.

It has been the general practice to determine nitrogen by difference, after the successive removal and measurement of the other gases. The nitrogen figure is thus liable to an accumulation of errors, due to physical solubility, leakage, and, to some extent, reading error, in the determination of each of these constituents.

For many purposes this is not important; in some cases, however, such as the analysis of the gaseous products of the decomposition of nitro-explosives, a more refined method has been desirable.

For mixtures in which the only constituents other than nitrogen are acid gases and oxygen, these can be simply and accurately removed in one manipulation by means of such absorbents as alkaline pyrogallol. For mixtures containing hydrogen, carbon monoxide or hydrocarbons, the problem is more complicated.

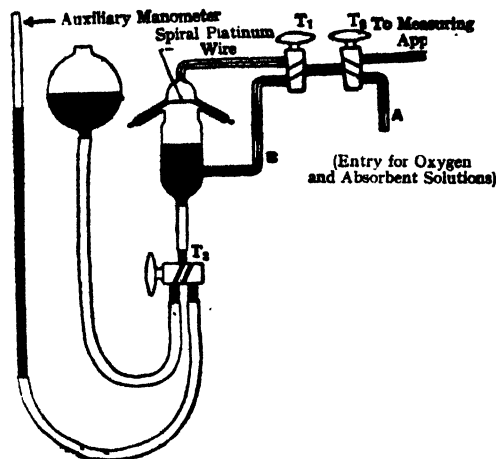
In the method described here, all gases other than nitrogen (with gases of the argon group) are removed with only one manipulation of the sample, by (i) burning the combustible constituents in a regulated stream of electrolytic oxygen, in the presence of alkali in a specially designed combustion vessel, and (ii) absorbing the excess of oxygen by introducing pyrogallol into the same vessel in the presence of the gas. The residual gas, consisting of nitrogen, is then directly measured.

**PROCEDURE.**—The combustion vessel is of the type described in a previous paper (*J. Sci. Instr.*, 1931, 8, 18).

The sample of gas, conveniently of about 30 c.c., is measured in an apparatus connected with the combustion vessel. The apparatus used is of the general

type previously described (ANALYST, 1929, 54, 517), but is of greater capacity, and is fitted with the special combustion vessel in place of the bulb, B, and reservoir, R, (*loc. cit.*).

The gas is transferred to the combustion vessel, into which about 5 c.c. of 40 per cent. potassium hydroxide solution have previously been introduced. The gas is followed by mercury, past the tap of the combustion vessel to the end of the capillary, and is thereafter kept under slightly positive pressure. Possibility of leaks in or out through the tap, T<sub>1</sub>, is thus eliminated.



The platinum wire is now heated electrically to a bright red heat, and a slow stream (about 2 bubbles per second) of electrolytic oxygen is bubbled in through the side tube, B. The amount of oxygen so added need not be measured, neither does the presence of hydrogen in it matter. If methane is present in quantities above 2 or 3 per cent. the wire should be maintained at yellow heat. In the absence of methane, it is practicable to proceed with the wire at dull red heat, but the action is slower than at bright red heat, and the rate of oxygen inflow must be decreased. As the action goes on, the temperature of the wire increases, owing to the decrease in the thermal conductivity of the gas mixture as hydrogen disappears; care should be taken that the wire does not become unduly hot in this way.

As oxygen passes into the combustion vessel a steady decrease of volume is observed, corresponding with the removal of combustible gases. When the volume reaches a minimum and begins to increase again, it indicates that oxygen is now being added in excess. A sensitive and convenient device for checking whether the amount of gas in the combustion vessel is increasing or decreasing is the auxiliary manometer described in a previous paper (ANALYST, 1929, 54, 521). This consists of a manometer which can be connected with the bottom of the combustion vessel by reversing a 3-way tap, T<sub>3</sub>. A further indication that sufficient oxygen has been added is that, when combustion ceases, the temperature of the wire abruptly falls; a voltmeter connected across the platinum coil can serve as a resistance thermometer and will give a sensitive indication of this.

After a slight excess of oxygen has been added, the wire, if it has not already been so heated, should be brought to bright yellow heat for two minutes to ensure the combustion of any small proportions of methane. The vessel is then allowed to cool for about two minutes, and a small quantity of aqueous pyrogallol solution is introduced from A. A suitable pyrogallol solution to use is one of about 25 per cent. strength and equal in volume to that of the alkali already taken.

It is my experience that any error due to evolution of carbon monoxide from the pyrogallate is less than 0.05 per cent. of the total gas. If, however, there is any suspicion that such evolution has been appreciable, a test and correction may be made subsequently, by adding a small quantity of oxygen and heating the platinum wire at dull red heat for one minute. If there is a little alkali in the vessel, 1 volume of carbon monoxide will give a contraction of 1.5 volume.

An alternative absorbent for oxygen, which can in no circumstances evolve carbon monoxide, consists of sodium hydroxide, sodium hydrosulphite and sodium anthraquinone- $\beta$ -sulphonate (Fieser, *J. Amer. Chem. Soc.*, 1924, 46, 2639; ANALYST, 1925, 50, 89). It is, however, slower than pyrogallol in its action, and the solution does not keep.

Absorption is usually complete within 15 minutes. The gas then consists only of the nitrogen (with gases of the argon group) which was in the original sample. This nitrogen is transferred to the measuring vessel and measured.

In transferring the gas to the measuring vessel, the reagent is brought only as far as the tap,  $T_1$ , which is then reversed, and the gas sample is followed into the measuring vessel by mercury from the side capillary B.

If incomplete absorption is suspected, a test may be subsequently applied by adding hydrogen and burning any residual oxygen by means of the hot wire (Ambler, ANALYST, 1930, 55, 677). The whole of the excess oxygen may, if desired, be determined by this method; it is quicker and more sensitive than the absorption method, but involves a larger number of manipulations and readings.

ACCURACY.—A high degree of accuracy is to be expected on the following grounds:—(1) Loss of the sample by leakage, once it has been transferred to the combustion pipette, is impossible. (2) Similarly, if the pressure in the combustion vessel is kept positive, leakage inwards is impossible. (3) The gas sample is transferred from one vessel to another only twice in the determination. (4) Only two readings are taken in the determination. (5) The gas comes in contact with a liquid reagent only once, and this reagent is a strong solution of relatively small volume. Hence, error due to solution of nitrogen in water is reduced to a minimum.

The following analyses show the consistency of the results obtained:

Percentage of nitrogen found:

			Sample I.	Sample II.
Determination I	..	..	32.04	15.94
II	..	..	32.00	15.99
III	..	..	32.10	

Experiments, in which a known amount of nitrogen was present, gave results as follows:

			Sample I. Per Cent.	Sample II. Per Cent.
Nitrogen taken	..	..	83.65	64.91
Nitrogen found	..	..	83.58	64.93

The work arose from a discussion with Mr. T. C. Sutton, who emphasised the advantages of developing a more direct method of determining nitrogen in the products of explosives than the difference methods hitherto in use.

RESEARCH DEPARTMENT, WOOLWICH.

## Notes.

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

### THE COLORIMETRIC DETERMINATION OF CHLORATES BY THE USE OF ANILINE HYDROCHLORIDE.

ON several occasions I have used the aniline method for determining small quantities of chlorate ion. A recent supply of aniline, however, failed to give the reaction satisfactorily, the colour developing but fading in a few seconds.

Redistillation and treatment with various reagents, in the hope of removing a deleterious impurity, were of no avail. The following simple expedient, however, afforded a reagent which gave a colour of the usual degree of permanence.

The aniline, which had been freshly distilled to remove colour, was made into the hydrochloride reagent in the usual manner, and diluted to within about 2 ml. of the final volume. One or two drops of a strong solution of potassium chlorate were added and the liquids thoroughly mixed, so that a slight, but permanent, blue colour remained. Dilution was then completed.

After the elapse of sufficient time at room temperature for the precipitate to clot (which requires 18 hours or more) the liquid was filtered, and the resulting colourless filtrate was then used as the reagent.

Further work on the nature of the impurity causing this effect is in progress.

R. A. JONES.

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### BELL'S "SALE OF FOOD AND DRUGS."

#### ERROR IN SCHEDULE OF PERMITTED PRESERVATIVES.

ATTENTION has been drawn to an error in the First Schedule to the Public Health (Preservatives, &c., in Food) Regulations, as printed on p. 211 of Bell's "Sale of Food and Drugs," 8th Edition. The permissible quantity for sulphur dioxide in sugar, etc., in par. 8, is 70 parts per million, not 370 parts, as stated.

EDITOR.



## Notes from the Reports of Public Analysts.

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

### CITY OF LEEDS.

#### ANNUAL REPORT OF THE CITY ANALYST FOR THE YEAR 1930.

OF the 2936 samples examined, 2123 were foods and drugs, 67 being informal samples (5 adulterated) and 2056 formal samples (218 adulterated). Of the 1530 samples of milk examined, 182 were adulterated.

**SULPHUR DIOXIDE IN POTTED MEAT.**—Four of the 11 samples examined were adulterated. Two of them contained 2 per cent. and 8 per cent. of starch, respectively, and the vendor in the second case was warned by letter from the Medical Officer of Health. Each of the other 2 contained 20 parts of sulphur dioxide per million, but it was decided not to take proceedings against the retailers concerned, as there was no evidence that the preservative had been added by the retailers in question. The explanation given was that the potted meat had been prepared from corned beef, and, on samples of this being taken, it was found that they all contained sulphur dioxide to the extent of 15 parts per million. The presence of this is probably due to the use of preserved gelatin in the course of manufacture. If so, the articles in question are covered by Section 4 (1) (ii) of the Public Health (Preservatives, etc., in Food) Regulations, 1925-27.

**RUM PUNCH WITHOUT RUM.**—One sample was taken and found to contain no rum, being a non-alcoholic liquor possessing the properties of a cordial. The manufacturers were warned by letter from the Medical Officer of Health.

**SWEET SPIRITS OF NITRE.**—Two of 5 samples did not conform with the requirements of the British Pharmacopoeia, 1914, as regards composition. One contained no ethyl nitrite, being an imitation mixture consisting of nitre, sugar, ammonium acetate, alcohol, and water; the other contained only 1.08 per cent. of ethyl nitrite, instead of the 1.52 per cent. minimum. In the first case the manufacturers were warned by letter from the Medical Officer of Health, whilst in the second case the retailer was summoned, the summons being dismissed, under the Probation of Offenders Act, on payment of 14s. 6d. costs.

**ENAMEL AS SUSPECTED CAUSE OF DERMATITIS.**—A sample of black enamel suspected of causing dermatitis of the hands of a painter was found to consist of a bituminous basis dissolved in "Westrosol" (trichloroethylene).

**CRYSTALS IN TINNED SALMON.**—Crystals of magnesium ammonium phosphate were discovered in a tin of salmon; they had probably been formed by the chilling of the salmon juices when in cold storage.

C. H. MANLEY.

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## Legal Notes.

*Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.*

### GINGELLY (SESAME) SEEDS IN RASPBERRY JAM.

ON November 14th a grocer was summoned at Tower Bridge Police Court, at the instance of the Bermondsey Borough Council, for selling a pound pot of raspberry jam to the prejudice of the purchaser.

An inspector of the Borough Council stated that he had bought a pound pot of raspberry jam from the defendant for 5d. It was labelled "Pure Preserves. Raspberry with a small addition of Fruit Juice."

The certificate of the Public Analyst (Mr. A. P. Davson, F.I.C.) showed that the jam contained only 22 per cent. of raspberries, 21 per cent. of apple pulp, and 175 gingelly seeds to the ounce of jam.

The inspector said that the seeds came from Czechoslovakia, where their use was the production of oil by crushing. If the seeds were bitten they would impart an oily taste to the jam. In reply to the magistrate, he said that the object of putting the seeds into the jam was to suggest that it was full of raspberries, since, from the layman's point of view, these seeds resembled raspberry seeds both in shape and size.

The defendant pleaded a warranty, and the summons was dismissed. A summons against the manufacturer was applied for and granted.

## Report of the Government Chemist upon the Work of the Government Laboratory.

FOR THE YEAR ENDING 31st MARCH, 1931.\*

DURING the year 1930 to 1931 the Government Laboratory has carried out work for the same Government Departments as previously (ANALYST, 1930, 55, 689), and the total number of samples examined was 517,462, a decrease of 27,960 on the previous year, which reflects the present depression in industry generally.

MINISTRY OF AGRICULTURE AND FISHERIES.—*Butter and Margarine*.—Ten of 831 samples of butter, and 10 of 344 samples of margarine contained over 16 per cent. of water. Two samples of margarine contained over 10 per cent. of milk-fat.

*Cheese*.—The percentages of water and fat showed that 46 per cent. of the samples had been prepared from whole milk, 34 per cent. from milk containing three-quarters to the whole of its fat, 11 per cent. from milk containing from one-half

\* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Price 9d. net.

to three-quarters of its fat, and 9 per cent. from milk containing from one quarter to one-half of its fat.

*Cream.*—One group of the 133 samples, representing 7 per cent. of the total, contained 45 to 55 per cent. of fat, and the other group 19 to 35 per cent.

*Sheep Dips.*—Of 114 samples, 20 were reported defective.

*Fertilisers and Feeding Stuffs Acts.*—Of 3 fertilisers examined, one contained one-fiftieth of the declared quantity of phosphoric acid, and reversion of soluble phosphate, probably caused by contamination with calcium carbonate in the mill, had reduced it below the allowed limit. A bone meal fertiliser consisted of vitriolised bone substitute, which contained less phosphoric acid than the statutory declaration signified. Of 8 feeding stuffs, deficiencies in excess of the allowed limits for oil or protein, or both, occurred in a laying meal, a biscuit meal and two meat meals; a ground oats sample contained 10 per cent. of barley; a barley meal contained an excess of sand; a meat and bone meal contained excess of salt, and a maize meal, containing a lower proportion of protein, had been sold as maize gluten meal. In all these cases the results of the agricultural analysts were confirmed.

*Miscellaneous Articles.*—Of the eggs suspected to be on sale without proper marking, 12 afforded evidence of preservation in silicate of soda and 7 in lime water, and 13 eggs had been treated to remove the original markings of origin. Under the Agricultural Produce (Grading and Marking) (Wheat Flour) Regulations, 91 plain, 19 self-raising, 55 Yeoman flours, 11 samples of wheat, and 20 of wheat meal were tested; under the Agricultural Produce (Grading and Marking) Cider Regulations, 14 samples of cider were examined for grading as select cider (Champagne process) and select cider, respectively; and 88 samples of honey from English and Welsh sources were examined to obtain their characteristic features.

*CUSTOMS AND EXCISE.—Beer.*—The total number of samples examined in connection with duty on beer was 52,423, a decrease of 7249 on the previous year. The original gravity of the wort was declared by the brewers 1 to 5 degrees low in 178 cases, where 6536 samples were taken. In 166 cases of 1922 samples of beer as retailed, dilution had occurred; in 10 cases this was equivalent to additions of over 4 gallons of water per barrel. Fifty-one of 1803 samples of beer, wort, malt, etc., contained arsenic in slight excess of the limit. *Cocoa and Chocolates.*—To assess duty or drawback 15,459 samples, 11,306 from imported, and 4153 from exported, foods were examined. The *Hydrocarbon Oils Duty* necessitated the analysis of 10,219 samples. *Hydrometers, Saccharometers, Thermometers and Graduated Vessels.*—The increased production of alcohol of high strength has caused the extension of the range of spirit hydrometers, and a new Sikes "B" hydrometer has been devised capable of indicating the highest alcoholic strengths at all temperatures likely to be experienced in this country. A few saccharometers and hydrometers are being prepared for trial, plated with nickel and chromium, instead of brass plated with gold, to see if they will be capable of giving more accurate determinations over a longer period.

*Spirits.*—The export samples of gin and liquors for tests of the spirit strength and character to check claims for drawback numbered 2170, and, in addition, 1976 of these samples were tested for sugar. The strength or quantity of sugar had been over-stated in 150 cases. Tests for "obscuration" are now being made on these samples, *v.e.* the apparent reduction in strength due to influences of substances in solution. For assessment of duty or drawback 71,782 samples of Sugar were examined, 403 of Glucose, and 545 of glucose for export. Of beet pulp, juice, molasses and refined sugar, 4208 samples were examined. Of Tea, 206 samples of 30,235 were reported against, 136 on account of foreign substances,

and 70 as unfit for human consumption. *Tobacco*.—To see that the limits of 32 per cent. of water and 4 per cent. of oil were adhered to in manufactured tobacco, 8783 samples were examined. Fourteen per cent. of moisture is fixed for drawback purposes for export tobacco and snuff, and 83,356 samples, in connection with 69,943 certificates, were taken. Moisture, inorganic matter, sand, and extraneous matter have to be determined for offal tobacco for export, and for this purpose 44,462 samples were taken.

**MINISTRY OF HEALTH.**—Thirty-nine samples of condensed milk were reported against, mostly in connection with labelling, but in two cases the milk was below the minimum standard, and in eight the quantity of whole milk represented on the tin was overstated. Forty-six samples of fruit, canned vegetables, chocolate, liquid eggs, etc., were reported to the Board of Customs and Excise as contravening the regulations, 28 for containing sulphur dioxide, two for benzoic acid contrary to the regulations, or in excess of the amount allowed, and three for benzoic acid and sulphur dioxide. Three samples of tinned vegetables contained copper, 3 of butter and 3 of margarine contained boron preservative, and 4 of brown refined sugar contained formic acid; it was stated that this had been produced in refining and had not been added.

**FOOD AND DRUGS ACT.**—Twenty samples of food were examined as samples referred by magistrates; 12 were milks alleged to be deficient in fat or non-fatty-solids, three peppers containing turmeric, one sausage alleged to be deficient in meat, and another sausage said to contain excess of sulphur dioxide; cream containing boric acid, and whisky and gin said to contain excess of water. The 3 cases of disagreement with analyses put forward by the prosecution were as follows:—Sulphur dioxide in sausage was within the prescribed limits, but diminution during storage could not be determined. Fifty-three per cent. of meat was found in a sausage alleged to contain less than 50 per cent., and 68.9 per cent. of proof spirit in a gin alleged to contain less than 65 per cent.

**HOME OFFICE.**—*Examination of Paint.*—A question having arisen as to the number of coats of paint applied to some buildings, small flakes of the paint were obtained, and transverse section were cut for microscopical and photo-micrographic examination.

*Examination of Soot.*—A sample of soot derived from burners using oil as fuel was examined as the result of a complaint that it had an irritant action on the mouth and throat of a worker engaged in cleaning the flues. It was found to contain free sulphuric acid.

**STATIONERY OFFICE.**—*Deterioration of Cinematograph Films.*—An investigation was undertaken into the causes of the deterioration of cinematograph films of national importance, and into the question of the restoration and preservation of such films. The celluloid base of the films was found to be in good condition, despite a little deterioration. It was not responsible for the damage and discoloration of the silver image of the picture. Retention of silver salts in the gelatin was the cause of most of the deterioration, and this retention arose from imperfect washing of the films, whereby "hypo" was left in the film, or to the use of weak or exhausted "hypo." No doubt, the reason for these defects lies in the rapidity with which it is frequently considered necessary to put films into circulation, but the inevitable result is to produce darkening and staining of the high lights or else dissolution of the residual silver, with subsequent blurring of the image. It was suggested that inefficient washing machines should be discarded, and improved methods of washing were advocated, especially incorporating the use of a final treatment with fresh "hypo" and subsequent washing. Apparatus for this purpose was inspected and approved before use in preparing better pictures from the films.

D. G. H.

## Straits Settlements.

### REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR 1930.

THE Government Analyst, Mr. J. C. Cowap, B.Sc., F.I.C., reports that, as in previous years, the greater portion of the routine work has been provided by the Monopolies Department and has consisted in the examination of opium, chandu, chandu dross, deleterious drugs, European and Chinese imported liquors, toddy and the products of the local distilleries. From the Medical Department have been received toxicological and clinical specimens, drugs, and samples of drinking waters and sewage effluents. For the Police Department there have been examined explosives, drugs, liquors, toxicological specimens, poisons and documents. From these three is derived practically the whole of the official work of the Analyst's Department.

The sources of the non-official work, *i.e.* work other than that undertaken on behalf of the Government of the Straits Settlements, are the Unfederated Malay States, the Municipality of Penang, the Admiralty and commercial firms. All this is revenue producing, and in it are included the testing of petroleum, the examination of tank air on ships which have carried petroleum, and the methylation of spirit.

**CHANDU DROSS.**—The number of packets of chandu dross examined on purchase by the Government from smokers has shown a remarkable decrease, the actual number examined being 9262, as against 25,577 in 1929. It appears that the present commercial depression has enhanced the value of such dross for re-smoking. Rigorous examination for dross adulteration and more drastic action against offenders in this regard have also tended to diminish the number of low-grade samples returned.

**LEAD POISONING FROM FACE POWDERS.**—Several strange cases of acute lead poisoning among Chinese women of good class were investigated. Many possible sources were examined, and eventually the cause was traced to face powders containing high percentages of lead; one, indeed, consisted of pure lead carbonate. Two samples analysed in Penang also consisted essentially of lead carbonate. Further investigations in Singapore and other Settlements disclosed similar cases. It was also demonstrated that such lead-containing face powders were in wide use among Chinese women.

At the end of the year legislation directed against the use of such cosmetics was under consideration.

**STANDARDS FOR TODDY.**—Of the 735 samples of toddy analysed, 67 contained added water, and 64 others were of poor quality. Although there are no legal standards for toddy, except as regards acidity and alcohol, a method of analysis has been agreed to by this Department and the chemists of the Federated Malay States, upon which departmental action can be taken.

**COPPER IN SPIRITS.**—Ninety-two samples of samsoo from local distilleries were examined for the presence of copper, and 32 of these were found to contain an excessive proportion of that metal. It is derived from the worms and vats of the distilling apparatus.

**POISONING CASES.**—Sixty-eight specimens of viscera, stomach washings, contents, etc., were received from the Medical Department, together with 12 associated exhibits. Poison was found in 47 cases. The poisons found were opium, tuba root, mercuric chloride, cyanide, alkali, alcohol, phenols, oil of winter-green, atropine, acids, strychnine, ground glass and kerosene.

In the 37 cases investigated for the Police Department the poisons found were luminal, opium, potassium cyanide, caustic soda, formic acid, and mercuric sulphide.

**VALUATION OF SAND.**—Analyses of 368 samples of sand were made for the Land Office. This was done in connection with a land acquisition case where it was claimed that certain sands had considerable value as being suitable for glass manufacture. Analysis showed that the sands were practically worthless for such a purpose. Largely as the result of these analyses, compensation claimed for such sand was reduced by the Court from over 1 million dollars to \$7500.

**MILK.**—Of 20 samples of milk purchased from licensed vendors in Penang Municipality, 10 were adulterated with water.

**LEAD IN BEAN CURD.**—Six samples of bean curd, imported in tins into Penang Municipality, contained an appreciable quantity of lead, due to the unsatisfactory soldering of the tins.

**DELETERIOUS DRUGS.**—One hundred and eight exhibits in 39 cases of suspected deleterious drugs were examined. These were mainly smuggled drugs or anti-opium medicines. The former consisted of heroin, morphine, cocaine, and ganja. Many of the anti-opium medicines contained morphine, heroin or opium.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

### Food and Drugs Analysis.

**Use of Selenium as Catalyst in the Determination of Nitrogen by the Kjeldahl Method.** M. F. Lauro. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 401-402.)—The author has found that the time required for the Kjeldahl digestion of flour, wheat, cottonseed meal, etc., using 25 c.c. of sulphuric acid, 10 gm. of potassium sulphate and 0.1 to 0.2 gm. of selenium as catalyst, was very considerably less than the time required under the same conditions of heating, using the same acid mixture, but with the usual copper sulphate or mercury catalyst instead of selenium. The table of results with the different catalysts shows that the time required with the selenium catalyst was in some cases less than one-third of the time required with the copper catalyst. No sodium or potassium sulphide need be added before the distillation, as required with the mercury catalyst.

S. G. C.

**Factors for Converting Percentages of Nitrogen in Foods and Feeds into Percentages of Proteins.** D. B. Jones. (*U.S. Dept. Agric. Circular No. 183*, 1931.)—The use of the constant factor 6.25 for converting the percentage of nitrogen in foodstuffs into percentage of protein involves two assumptions, neither of which is necessarily valid: that all the nitrogen is present as proteins, and that all proteins contain 16 per cent. of nitrogen. Determinations of the nitrogen contents of 121 different proteins isolated from vegetable and animal products gave values varying from 13 to over 19 per cent. The following factors

are suggested for converting the percentages of nitrogen in various substances into percentages of protein. It is not claimed that these factors will give absolutely correct values, but it is believed that they will yield results more accurate than those calculated on the basis of the 6.25 factor.

Substance.	Factor.	Substance.	Factor.
Cereal grains:		Oilseeds and nuts:	
Wheat, endosperm ..	5.70	Brazil nut .. ..	5.46
„ embryo ..	5.80	Hazelnut .. ..	5.30
„ bran ..	6.31	Walnut .. ..	5.30
„ whole kernel ..	5.83	Peanut .. ..	5.46
Rye .. ..	5.83	Soya bean .. ..	5.71
Barley .. ..	5.83	Butternut .. ..	5.30
Oats .. ..	5.83	Castor bean .. ..	5.30
Rice .. ..	5.95	Animal products:	
Maize .. ..	6.25	Milk .. ..	6.38
Oilseeds and nuts:		Eggs .. ..	6.25
Hempseed .. ..	5.30	Meats .. ..	6.25
Cottonseed .. ..	5.30	Gelatin .. ..	5.55
Sunflower seed ..	5.30	Leguminous seeds:	
Flax seed .. ..	5.30	Navy bean .. ..	6.25
Squash seed .. ..	5.30	Lima seed .. ..	6.25
Pumpkin seed .. ..	5.30	Mung bean .. ..	6.25
Sesame seed .. ..	5.30	Velvet bean .. ..	6.25
Canteloupe seed ..	5.30	Aduski bean .. ..	6.25
Almonds .. ..	5.18	Jack bean .. ..	6.25
Coconut .. ..	5.30		

T. H. P.

**Residual-Current Measurements in Control of Metal Solution in Milk.** H. T. Gebhardt and H. H. Sommer. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 414-418.)—The currents flowing between electrodes of copper and a more noble metal, *e.g.* gold or platinum, when immersed in milk under different conditions of temperature, acidity, concentration of oxygen, etc., have been measured. The assumption is made that the solution of metallic copper in milk occurs by electrochemical action, passive areas in the surface acting as cathodes, and that the action of these cathodes can be simulated by connecting specially cleaned copper with an artificial cathode of a more noble metal. It is stated that the currents produced give a qualitative measure of the rate of solution of copper in milk under the different conditions, and that the amount of copper dissolving may be calculated (from the Faraday equivalent), giving a fair approximation to the gravimetrically determined values. With nickel, the currents were observed to fluctuate with time and the surface condition of the metal more noticeably than with copper, but there was a parallelism between the current generated and the loss in weight of the nickel; the current measurements may thus give information concerning the rate of solution of nickel in milk in the same way as was found with copper. Aluminium, zinc, and tin, immersed in milk, when connected with the noble electrode, gave strong currents, although these metals actually dissolve in milk only to a slight extent; the currents produced, therefore, give no indication of the solubility of these metals in milk.

**Ferricyanide Method for the Determination of Reducing Sugars. Modification of the Hagedorn-Jensen-Hanes Technique.** A. C. Hulme and R. Narain. (*Biochem. J.*, 1931, 25, 1051-1061.)—The modification of the method of Hagedorn and Jensen (*Biochem. Z.*, 1923, 135, 46) for the determination of reducing sugars, introduced by Hanes (*Biochem. J.*, 1929, 23, 99; *ANALYST*, 1929, 54, 349), has the advantage over copper reduction methods that much smaller amounts of sugar can be accurately determined. Hanes used the method for the determination of glucose and maltose; only the latter sugar gave a linear relation between the amount of sugar and amount of ferricyanide reduced. Callow (*Biochem. J.*, 1930, 24, 57) applied the Hanes method to the determination of invert sugar, but the method has not been standardised for fructose. For a study of carbohydrate metabolism in green leaves, it was first necessary to investigate the relationship of sugar to ferricyanide for fructose and for varying mixtures of the reducing sugars (glucose, fructose and maltose). It was also hoped to find a set of conditions under which the relationship of sugar to ferricyanide would be linear for all the reducing sugars, and under which the total reducing power might be expected to be the simple sum of the reducing powers of the component sugars. This possibility has been realised by changing the volume alone. Hanes's technique for the actual oxidation of the sugar by ferricyanide and the titration of the excess of ferricyanide with thiosulphate was carefully followed, with the exception that the volume of sugar solution taken was 10 c.c. in place of 5 c.c. The thiosulphate solution (approximately  $N/75$ ) was standardised against standard potassium iodate, and its strength was checked every 2 or 3 days, especially in warm weather. Two different methods for delivery of the required amounts of sugar in solution were used: (a) Sugar solutions of 0.01 and 0.05 per cent. concentration were prepared and transferred to the boiling tubes containing the ferricyanide from a 10 c.c. burette (graduated to  $1/50$  c.c. and fitted with a fine waxed jet) in the required quantities. (b) Sugar solutions of 0.01, 0.02, 0.025 and 0.05 per cent. were prepared and transferred to the boiling-tubes by means of accurate 2, 5 and 10 c.c. pipettes. In both cases the final volumes of sugar solution were made up to 10 c.c. with distilled water by means of a graduated pipette, when necessary. Water volume differences of 0.01 c.c. had no measurable effect on the results. Methods (a) and (b) were used indiscriminately, although in most cases duplicate determinations by means of the two methods were carried out, good agreement being obtained. In each case the total number of determinations was four and the maximum variation in c.c. of thiosulphate solution ( $N/75$ ) was 0.03 c.c. This modification is shown to give a linear relation between the amount of sugar and the amount of ferricyanide reduced over a range of 0.2 to 3.0 mgrms. in the case of glucose, fructose and invert sugar, and of 0.2 to 3.5 mgrms. in the case of maltose. By statistical treatment the linear relation was found to be accurate to 0.0067 mgrm. sugar (mean value for the four sugars), i.e. 0.5 per cent. of the mean amount of the sugars determined. Expressing the relation between amount of sugar and the amount of ferricyanide reduced in terms of  $N/100$  thiosulphate, the following relation was found:—Sugar =  $b$  (observed thiosulphate +  $a$ ), where  $a$  has the value 0.05 c.c.  $N/100$  thiosulphate



for all the sugars investigated; factor *b* is shown to be, for glucose 0.340, for fructose 0.341, for invert sugar (prepared by hydrochloric acid inversion of sucrose) 0.338, and for maltose 0.455. In a mixture of sugars the ferricyanide reducing power of one sugar is unaffected by the presence of the others. The presence of sucrose does not affect the reducing value of the other sugars present in a mixture. Invert sugar, prepared by acid hydrolysis of sucrose, has a slightly higher reducing value than a synthetic mixture composed of equal amounts of glucose and fructose. For the determination of sucrose, inversion by 2.5 per cent. hydrochloric acid for 12 minutes at 60° C., as suggested by Hinton and Macara (*ANALYST*, 1927, 51, 668), followed by determination of ferricyanide reducing power, gives accurate results; citric acid inversion gives results which are too high. The application of the ferricyanide reduction method to the determination of individual sugars in plant extracts is discussed.

P. H. P.

**Glyceride Structure of Beef Tallows.** A. Banks and T. P. Hilditch. (*Biochem. J.*, 1931, 25, 1168–1182.)—Four beef tallows from various North and South American sources have been investigated with reference to their content of fully-saturated glycerides and the component fatty acids of the latter and of the whole fats. In the whole fats the united molar percentage of stearic, oleic and linolic acids tends towards one of two definite figures (about 62 or about 69 mols. per cent.), in spite of wide variations in the amount of stearic acid, and correspondingly of oleic (with linolic) acid. This relation also subsists in a number of other tallows for which previous analyses are available. The proportion of fully-saturated glycerides present varies according to the relative total amounts of saturated and unsaturated acid in the whole fats, but the composition of the acids present therein tends to much the same figure in all cases, namely, about 60 per cent. of palmitic (with myristic) acid and about 40 per cent. of stearic acid. These regularities in structure, which are strikingly similar to those recently observed by Hilditch and Sleightholme (*Biochem. J.*, 1931, 25, 507) in a series of butter fats, are discussed in regard to their possible implications concerning the processes involved in the elaboration of storage fat in the animal. It is hoped to obtain further insight into the problems discussed from a parallel study, at present in progress, of the body-fats of the pig, in which the fats from a single animal fed on a controlled diet are being investigated.

P. H. P.

**Composition of Philippine Kapok Seed Oil.** A. O. Cruz and A. P. West. (*Philippine J. Sci.*, 1931, 46, 131–137.)—The pods of the Philippine kapok tree (*Ceiba pentandra* Gaertner) consist of approximately 51 per cent. of husk and core, 32 per cent. of seeds, and 17 per cent. of floss, one pod weighing about 32 grms., and giving an average of 149 seeds weighing 10 grms. The seeds yielded 25 per cent. of oil, which, after clarification, had the following characteristics: Sp. gr. at 30°/4° C., 0.9109;  $n_D^{20}$  C., 1.4678; saponification value, 192.1; iodine value (Hanus), 95.6; unsaponifiable matter, 0.78 per cent.; acid value, 7.39; saturated acids (corrected), 18.64 per cent.; unsaturated acids (corrected), 75.71 per cent.; iodine

value of unsaturated acids plus unsaponifiable matter, 123.4; iodine value of unsaponifiable matter, 82.4. The unsaturated acids were separated by the lead salt and ether method, and converted into their bromine derivatives. No ether-insoluble hexabromide was obtained, showing the absence of linolenic acid. The saturated acids were separated, esterified with methyl alcohol and dissolved in ether, the ethereal solution was washed and dehydrated, and the impure esters were distilled under reduced pressure and subsequently redistilled, and the amounts of the individual acids were calculated. The composition of the oil, expressed as glycerides of the acids, was: oleic, 49.8; linolic, 29.3; myristic, 0.5; palmitic, 15.9; stearic, 2.3; arachidic, 0.8; unsaponifiable matter, 0.8 per cent. The percentage of linolic and palmitic glycerides is slightly lower in kapok oil than in cottonseed oil.

D. G. H.

#### Quantitative Determination of some of the Constituents of Rhubarb.

P. Valaer. (*Amer. J. Pharm.*, 1931, 103, 495-503.)—The efficiency of preparations of rhubarb is reduced by the addition of alkalis, such as is carried out in many Italian preparations. The method used by the American laboratory of the Bureau of Industrial Alcohol for determining the rhubarb in fluid extracts and other preparations is based on the Bornträger reaction (*Z. anal. Chem.*, 1880, 19, 165), and, under certain carefully controlled conditions, the depth of colour extracted by the ether from the acid extract, and the colour developed on treatment with ammonia, vary almost directly in proportion to the amount of rhubarb or other emodin-bearing drugs present. By these readings the free emodin bodies are estimated, and a separate portion of the preparation is then hydrolysed with concentrated sulphuric acid and the process repeated, whereby the total emodin readings are obtained, representing the colour due to free and combined anthraquinone bodies. The fluid extracts were prepared to contain 15 minims of the U.S.P. fluid extract per fluid ounce in approximately 45 per cent. alcohol, and the free emodin was determined by extracting 25 c.c., acidified with 2 c.c. of 0.1 *N* sulphuric acid for 5 minutes with 50 c.c. of ether, drawing off the aqueous liquor several times, and placing 10 c.c. of the yellow ethereal extract and 10 c.c. of concentrated ammonia in a Nessler tube, and, after mixing, leaving for two hours. The colour of the remainder of the extract is read in a 1/16 inch Lovibond cell, and the mixture in the Nessler tube is made up to 50 c.c. with water, the ether flicked off the top, and the red colour read in a 1/8 inch cell. To find the total emodin, 25 c.c. of the preparation, made as described above, are treated with 2 c.c. of concentrated sulphuric acid, shaken, left overnight, refluxed for 30 minutes with a tube condenser, shaken in a separating funnel with 50 c.c. of ether for 5 minutes; after settling, the aqueous layer is drawn off. This is repeated several times, and the colour readings of the extract are determined as before. The average colours observed were 5.4 yellow in 1/16 inch cell, and 5.4 red in 1/8 inch cell before hydrolysis, and 12 yellow and 10 red after hydrolysis, the ratio of yellow to red being 1 to 1 before hydrolysis, and 6 to 5 after hydrolysis. It is important to determine the amount of alcohol necessary to keep the fluid extract in solution

and to prevent its deterioration while in solution, and it appears that about 45 per cent. of alcohol must be allowed in medicines where extract of rhubarb is used, or an allowance may be made on analysis. A second method is described, whereby the acidified extract is boiled with chloroform, and the chloroform extract is washed free from anthraquinones, and treated with sodium hydroxide, followed by water. Concentrated hydrochloric acid is then added to the alkaline solutions and washings, the mixture is extracted five times with chloroform, the extract is washed with water, allowed to settle, filtered and evaporated from a weighed vessel, and the residue is dried and weighed. The residue is then heated with potassium hydroxide solution, washed into a graduated flask, made up to volume, an aliquot portion is diluted with water, and the red colour is matched in a 1/8 inch cell. Hydrochloric acid is added in excess to another portion of alkaline liquid, which is then extracted with ether, and the yellow colour is matched in a 1/16 inch cell.

D. G. H.

**Determination of the Alkaloid Bases of Calabar Beans.** M. Polonovski and J. Cappelaere. (*J. Pharm. Chim.*, 1931, 123, 328-337.)—The determination of an isolated genalkaloid does not present any particular difficulty, as the transformation of the tertiary base into aminoxide only affects the alkalinity, and the reaction of Bertrand (*Bull. Soc. Chim.*, 1890, 21, 434) may be used to determine these alkaloids, since their silicotungstates are nearly as insoluble in the hydrochloric acid as those of the alkaloid bases. To the sample (sufficient to give a final weight of 0.1 or 0.2 gm.) are added 20 c.c. of dilute (4 : 1000) hydrochloric acid, and the whole is boiled. The 5 per cent. aqueous solution of silicotungstic acid is then added, drop by drop, until in slight excess, and heat is applied until the precipitate has collected at the bottom of the flask. After standing overnight the liquid is repeatedly filtered through ashless papers, and the precipitate is washed with the minimum quantity of slightly acid (hydrochloric) water, dried, and weighed. Eserine silicotungstate ( $\text{SiO}_2 \cdot 12\text{WO}_3 \cdot 2\text{C}_{15}\text{H}_{21}\text{O}_2\text{N}_3 \cdot n\text{H}_2\text{O}$ ) crystallises in chocolate-brown needles (m.pt. 197°-198° C.), and the geneserine salt, which is of a similar colour, blackens at about 182° C. and melts at 244° C. The m.pt.s. and characteristics of the silicotungstates of the Calabar bean alkaloids and several other alkaloids are given, together with the following table, showing the amounts of the residue of  $\text{SiO}_2$ ,  $12\text{WO}_3$  corresponding with 1 gm. of alkaloid:

				Theoretical.	In practice.	
Eserine	..	..	0.193	Geneserine	0.409	0.406
Strychnine	..	..	0.470	Genostrychnine	0.4922	0.495
Scopolamine	..	..	0.426	Genoscopolamine	0.4486	0.455
Atropine	..	..	0.4066	Genatropine	0.4289	0.430
Hyoscyamine	..	..	0.4066	Genhyoscyamine	0.4289	0.430
Morphine	..	..	0.4068	Genomorphine	0.4233	0.425

The genalkaloids may be determined volumetrically by Jonescu-Matiu's application of Votoček and Kašpárek's mercuric iodide method (*ANALYST*, 1927, 52, 100), whereby the solution to be determined (containing not more than 0.5 gm. of

the genalkaloid) and 5 c.c. of Valser's reagent (10 grms. of potassium iodide, 15 grms. mercuric iodide, made up to 100 c.c.) are mixed, and, after 5 minutes, centrifuged. The supernatant liquid is decanted, the precipitate is washed several times with 5 c.c. of 1 per cent. sulphuric acid, 10 c.c. of a mixture of 1 part of nitric acid and 2 parts of sulphuric acid are added, and the whole is heated until the precipitate is dissolved. The liquid is then poured into 15 c.c. of the acid mixture and made up to 230 c.c. with water, and *N*/10 potassium permanganate solution is run in until the colour persists for 1 minute. The addition of 10 drops of 10 per cent. sodium nitroprusside solution produces cloudiness, and *N*/10 sodium chloride solution is added, drop by drop, until the liquid is clear. The number of c.c. of sodium chloride solution is multiplied by the factor for the particular base, to obtain the quantity of genalkaloid taken, and the following table of these factors is given:

Alkaloid.				Theoretical.	In practice.
Strychnine	..	..	..	0.0153	0.01712
Scopolamine	..	..	..	0.0101	0.0114
Atropine	..	..	..	0.0097	0.0098
Hyoscyamine	..	..	..	0.0097	0.0098
Morphine	..	..	..	0.00957	0.0111
Eserine	..	..	..	0.00924	0.0093
Genalkaloid.					
Genostrychnine	..	..	..	0.01762	0.01767
Genoscolamine	..	..	..	0.01071	0.012
Genatropine	..	..	..	0.01024	0.01033
Genhyoscyamine	..	..	..	0.01024	0.01033
Genomorphine	..	..	..	0.0101	0.0113
Geneserine	..	..	..	0.00977	0.00987

An example is taken in detail of the determination of a mixture of eserine and geneserine, and the methods given above were found to be applicable to their determination in the Calabar bean.

D. G. H.

**Scopoletine Reaction for *Hyoscyamus* Extracts.** E. C. M. J. Hollman. (*Pharm. Weekblad*, 1931, 68, 1063–1068.)—The following test for scopoletine in tinctures of *Hyoscyamus* leaves (Dutch Pharmacopoeia, Edition V) has been examined. An aqueous extract of the residue from the evaporation of 5 c.c. of sample (or 100 mgrms. of extract) is shaken with 10 c.c. of ether, and the ethereal layer is shaken with 5 c.c. of water containing 2 drops of ammonia. In the presence of scopoletine a blue fluorescence appears in the water. The reaction was not obtained from *hyoscyamus* extracts prepared carefully by the author from native leaves of different origin, but was given by certain foreign leaves. A similar fluorescence, visible with difficulty with the help of a Tyndall cone, was obtained with stramonium extracts, and in one case it was apparent to the naked eye on dilution of the aqueous layer. Chlorophyll, which is also found in the aqueous layer, does not necessarily inhibit the reaction, though, since it produces a reddish fluorescence complementary in colour to that of the scopoletine, its removal is often desirable. Examination of the fluorescence in diffused daylight enables

15 per cent. of belladonna extract to be detected in *hyoscyamus* extract (1 per cent. in direct sunlight); the use of a lens was seldom satisfactory. Addition of a drop of dilute hydrochloric acid, which is supposed to stimulate the transference of the scopoletine to the ether, was found to have no appreciable advantage, so far as this purpose was concerned. It may, however, assist the removal (by filtration) of interfering leaf-substances, and, if it is used, a corresponding extra quantity of ammonia should be added at the final stage. J. G.

## Biochemical

**Some Biochemical and Physiological Aspects of Copper in Animal Nutrition.** I. J. Cunningham. (*Biochem. J.*, 1931, 25, 1267-1294.)—It was claimed by Waddell *et al.* (*J. Biol. Chem.*, 1928, 77, 769) that copper is essential for the utilisation of iron in haemoglobin building. An investigation was undertaken with a view to obtaining further information on this and cognate subjects. Determinations of copper were made in plants, foodstuffs, insects and marine animals. A systematic survey was also made of the occurrence of copper in the organs of twelve species of land animals (foetal, newly-born and adult). The results showed that the copper content of the skin and of the livers of rats is increased in relatively small degree compared with the additional copper given in the diet. Feeding the mother with additional copper caused increased storage of copper in the newly-born rat. Copper has a catalytic effect on the oxidation of "dopa" to melanin. "Dopa" (1-3:4-dihydroxyphenylalanine) is a chemical precursor of melanin, into which it is converted on oxidation. There is a high proportion and amount of copper in the ink sac of the octopus, but the ink itself contains no appreciable quantity of this element; it is suggested that the copper in the ink sac has a function in the formation of the ink, since the ink of such sea animals contains melanin. Copper feeding has little or no effect on the storage of iron in the foetal rat or in the hen's egg. Iron feeding has no effect on the storage or excretion of copper by the rat. The use of milk diets for production of a nutritional anaemia in rats is criticised. The stimulating effect of copper on the formation of haemoglobin in rats is confirmed, and a possible mechanism is suggested for its action. Feeding rats with high amounts of copper sulphate or copper acetate has no effect on their growth and reproduction, but high amounts of verdigris were observed to stop reproduction. Rats receiving one-fifth of the normal amount of copper in the diet will grow and reproduce normally. P. H. P.

**Soluble Enzymes Secreted by Hymenomycetic Mushrooms. Comparison of the Antioxygenic Power of Tannin and of the Phenolic Constituents of Essential Oils.** L. Lutz. (*Compt. rend.*, 1931, 193, 608-609.)—To tubes containing 5 c.c. of suitable culture medium were added, (a) 5 drops of a 1 per cent. solution of tannin; (b) 1 drop of a 1 per cent. solution or aqueous suspension of thymol, carvacrol, guaiacol, eugenol, anethole, methyl salicylate, and *m*- and *p*-cresol (obtained from essential oils); (c) both tannin and phenol as in (a) and (b). On seeding with *Stereum hirsutum*, *S. purpureum* or *Coriolus versicolor* there was in no case any change in the rate of oxidation of the tannin, as indicated

by its brown colour, though the oxidation of the phenol compounds was retarded. For example, guaiacol began to colour 3 days later, and *p*-cresol a month later than the control containing *C. versicolor* in the presence of tannin. The tannin thus appears to be a more powerful anti-oxidant than the phenols, to which it may act as a temporary protector against oxidation by monopolising the fungic oxidases. This is in accord with Moureu's theories of anti-oxygenic action.

J. G.

**New Method of Estimating the Activity of Peroxydases.** B. B. Dey and M. V. Sitharaman. (*J. Indian Chem. Soc.*, 1931, 8, 479-487.)—Existing methods are reviewed and are considered to give only approximate results. Thus, in the purpurogallin method high values may result from oxidation of the enzyme itself and the consequent precipitation of insoluble matter, and low results from the solubility of the purpurogallin in the acid added to stop the reaction. The rate of reaction in this method may vary widely (e.g. from 24 hours for beetroot to 7 days for milk) for peroxydases from different sources. The present method, which is stated to be free from these objections, depends on the conversion of hydroquinone into quinhydrone by peroxydase in the presence of hydrogen peroxide. The fruit sap is prepared from the frozen mass, obtained by storing thin slices of the fresh unripe fruit for 12 hours at  $-8^{\circ}\text{C}$ ., by expression in a hand-press, all turbidity then being removed by the centrifuge and by filtration through glass-wool. The final sap retains its activity for a week if stored at 0 to  $5^{\circ}\text{C}$ . with the addition of a few drops of toluene. Ten c.c. are digested with 10 c.c. of a fresh saturated solution of hydroquinone and 10 c.c. of 1 per cent. hydrogen peroxide at  $10^{\circ}\text{C}$ . for  $1\frac{1}{2}$  to 2 hours, after which time no more quinhydrone should be deposited. The precipitate is filtered off in an Allihn tube fitted with coarse asbestos below the perforated plate and fine asbestos above it (total thickness 5 mm.), washed with a cold 2 per cent. solution of hydroquinone, and then with 5 to 8 c.c. of ice-water, and dried until constant in weight (after about 48 hours) in a vacuum desiccator over sulphuric acid. The dry tube is reweighed after removal of the quinhydrone in a little absolute alcohol, insoluble impurities (1 to 2.5 mgrms.) being eliminated in this way. The solubility correction (21 to 23 mgrms.) is found by repetition of the experiment in the presence of a known amount of added quinhydrone in excess of that required to give a saturated solution. Alternatively, the precipitate may be removed in 10 c.c. and four 5 c.c. portions of alcohol, 20 c.c. of 95 per cent. alcohol, 20 c.c. of concentrated hydrochloric acid and 20 c.c. of 10 per cent. potassium iodide solution being added, the mixture immersed in ice-water and the iodine liberated and titrated immediately with 0.1 *N* sodium thiosulphate solution. This method is more rapid and gave more concordant results (for "Jhinga" juice); it avoids possible losses of quinhydrone on drying.

J. G.

**Comparison of Tests for Vitamin A in Cod-liver Oils.** K. H. Coward, F. J. Dyer, R. A. Morton and J. H. Gaddum. (*Biochem. J.*, 1931, 25, 1102-1120.)—Eleven samples of cod-liver oil and two concentrates have been examined biologically, chemically and physically for their vitamin A content, and a statistical

examination has been made of the results. The following values were determined: (1) Biological value; vitamin *A* content measured as described by Coward *et al.* (*Biochem. J.*, 1930, **24**, 1952; 1931, **25**, 551) by resumption of growth in rats whose reserves of this factor had been exhausted by being fed on a diet deficient in vitamin *A*. (2) Blue value; obtained by the action of antimony trichloride on the oil itself and measured by the Lovibond tintometer. (3) Blue value; obtained by the action of antimony trichloride on the unsaponifiable fraction of the oil and measured by the Lovibond tintometer. (4) Intensity of absorption at  $572m\mu$ , with the use of the blue solution obtained by the action of antimony trichloride on the oil itself, measured spectrophotometrically. (5) Intensity of absorption at  $606m\mu$ , with the use of the blue solution obtained by the action of antimony trichloride on the oil itself, measured spectrophotometrically. (6) Intensity of absorption at  $328m\mu$  for the oil itself, measured spectrophotometrically by the technique described by Morton and Heilbron (*Biochem. J.*, 1928, **22**, 987). Statistical examination of the results shows that: (a) The discrepancies between the physical and biological measurements are much larger than the known sampling error of the biological test; (b) of the physical and chemical measurements, the best agreement with the figures determined by the biological method was given by the measurement of the  $328m\mu$  absorption band, and nearly as good agreement was given by the Lovibond blue value of the unsaponifiable fraction of the oil; (c) both the  $572m\mu$  and  $606m\mu$  bands did not agree so well; (d) the Lovibond blue values obtained from the oils themselves give the least good agreement, and it would appear to be unwise to rely upon this method for the measure of vitamin *A* in an oil. It can only be used as a very crude indication of vitamin *A* potency. The results appear to indicate that certain oils may contain substances which increase the blue value, and other oils may contain substances which decrease the blue value. The oils whose biological values most closely agree with the values of the  $328m\mu$  band and the Lovibond blue values of the unsaponifiable fractions are those which were received direct from the manufacturers and examined within a few months of their preparation.

P. H. P.

**Antimony Trichloride Colour Test and the Ultra-Violet Absorption of Liver Oils and Concentrates.** A. E. Gillam and R. A. Morton. (*Biochem. J.*, 1931, **25**, 1346-1351).—Liver oils contain two independent chromogens which, with antimony trichloride, give coloured substances with absorption maxima at  $606m\mu$  and  $572m\mu$ , respectively. In concentrates these maxima are displaced to  $620m\mu$  and  $583m\mu$ . A comparison of ultra-violet absorption spectra with spectroscopic data on the colour test has shown (1) that the parallelism between the intensity of the  $606m\mu$  band and the intensity of the  $328m\mu$  band breaks down so seriously in extreme cases as to render it improbable that the  $606m\mu$  chromogen is vitamin *A*; (2) that the  $572m\mu$  chromogen and the substance responsible for the  $328m\mu$  band are probably identical, and (3) that the blue colour for rich oils and concentrates is often much deeper than would be expected on the basis of correlation between blue colour and ultra-violet absorption. Hence it is concluded that the matching

of blue colours with Lovibond glasses, though it may act as a rough guide to vitamin *A* potency, is theoretically unsound. The use of the  $328m\mu$  band as a quantitative criterion of vitamin *A* involves the measurement of the gross absorption at  $328m\mu$ , a magnitude, theoretically, including: (1) The true contribution of the  $328m\mu$  band, *plus* (2) the contribution of the materials always present in cod-liver oils, etc., *i.e.*, the normal fatty glycerides and the cholesterol, *plus* (3) the contribution of the variable and occasional non-vitamin *A* constituents, *e.g.*, free acids and other impurities. Hitherto in average medicinal cod-liver oils a correction term has been used for the joint effect of (2) and (3). Further experience on a wider range of materials has shown that the absorption of the fatty glycerides and the cholesterol is negligibly small, and that the excessively high values for the absorption at  $328m\mu$  arise from the presence of impurities which vary from sample to sample. The correction of the  $328m\mu$  intensity has, therefore, been abandoned as too uncertain in its operation. The authors have carefully explored the possibility that the intensity of absorption at  $328m\mu$  is a summation of two factors: one, the absorption arising from the  $572m\mu$  chromogen, and the other, that due to the  $606m\mu$  chromogen. The enquiry indicated that the substance responsible for the  $606m\mu$  band with antimony trichloride is very transparent to light of the wave-length near  $320-330m\mu$ .

P. H. P.

**Specificity in Tests for Vitamin A. New Conception of the Chromogenic Constituents of Fresh and Aged Liver Oils.** I. M. Heilbron, A. E. Gillam and R. A. Morton. (*Biochem. J.*, 1931, 25, 1352-1366.)—The ideal conditions for accurate colorimetric determinations are considered, and the actual characteristics of antimony trichloride as a colour-producing reagent are reviewed in detail. In a considerable number of oils characterised in the colour test by predominance of the  $572m\mu$  band over the  $606m\mu$  band, a large increase in the intensity of the latter band can be obtained by treatment of the oil beforehand with ozonised oxygen, hydrogen peroxide or benzoyl peroxide. Oils which initially show an excess of the  $572m\mu$  chromogen over the  $606m\mu$  chromogen undergo a slow spontaneous ageing, which results in a marked increase in the intensity of the  $606m\mu$  band. Increases in the  $606m\mu$  absorption are not at the expense of the  $572m\mu$  chromogen, and are not accompanied by similar increases in the latter, and the absorption at  $328m\mu$  remains practically constant throughout. The reality of the two chromogens and the possibility of recording the  $572-583m\mu$  band, even in oils and concentrates in which the  $606-620m\mu$  chromogen predominates, can be demonstrated visually and photographically by a modification of the ordinary procedure in the colour test, which suppresses or eliminates some of the  $606m\mu$  chromogen. The usual quantity (0.5 c.c.) of a solution of liver oil in chloroform is run into a test tube and a small quantity (0.3 c.c.) of the antimony reagent is added. The solution is then allowed to stand for 1 minute, when the remainder (4.7 c.c.) of the reagent is added, and the solution is examined spectroscopically. In nearly every case two separate absorption bands with maxima at  $565-575$  and  $600-620m\mu$  can be seen very clearly. The intensity of the former band is practically



the same as when the whole of the reagent is added at once, but the latter band is very much reduced in intensity. The bearing of the data on the vitamin *A* problem is discussed in detail, and the most plausible criteria for the vitamin are stated, namely, (1) a colourless or pale yellow substance exhibiting selective ultra-violet absorption free from fine structure and absorbing maximally at or near  $328m\mu$ , and (2) giving with antimony trichloride a coloured substance characterised by a maximum in the yellow between  $580$  and  $590m\mu$ , whilst the molecular extinction coefficients at  $328$  and  $580$ – $590m\mu$ , expressed in terms of vitamin concentration, should be approximately equal. The two main chromogens behave as if they were separate and distinct entities, but a close genetic relationship seems highly probable.

P. H. P.

**Vitamin A from Fish Livers.** P. Karrer, R. Morf and K. Schöpp. (*Helv. Chim. Acta*, 1931, 14, 1036–1040.)—Highly active vitamin *A* preparations may be obtained from the liver oil of *Hippoglossus* (halibut) by the following procedure: The oil, extracted from the liver by means of petroleum spirit of low boiling-point, is saponified with 12 per cent. solution of potassium hydroxide in alcohol at  $60^{\circ}$  C. in an atmosphere of nitrogen, the operation being complete after one hour's heating. Extraction of the unsaponified substance with petroleum spirit of low boiling point gives a product containing a large proportion of sterols. After evaporation of the solvent, the residue is dissolved in hot methanol and the solution kept at  $-15^{\circ}$  C. for some hours, almost the whole of the sterols then crystallising out. The solid is separated by filtering at  $-15^{\circ}$  C. with the help of a pump, and a further small amount of sterols is removed by concentrating the clear filtrate, and again cooling and filtering it. The product is rendered still purer and more active: (1) By cooling its methanol solution to  $-60^{\circ}$  C. and filtering or centrifuging at the same temperature, and (2) by subjecting its petroleum spirit solution to two successive fractional adsorptions with absorbent earth (Merck's Faser-tonerde).

The resulting pale yellow, viscous oil dissolves readily in ordinary organic solvents, undergoes oxidation in the air, and, when tested by the Carr-Price reaction, shows an activity of 10500 C.L.O. units. With rats on a diet free from vitamin *A*, 0.005 mgrm. per day per animal causes normal growth. The preparation is thus at least ten times as active as Suzuki and Nakimaya's biosterin, the most active vitamin *A* product previously obtained. The results show that the Carr-Price reaction and effect on growth follow approximately parallel courses. The carbon and hydrogen contents of the product are 83 to 84 and 10.5 per cent., respectively, and oxygen is also present. The molecular weight in camphor solution is 320. The preparation may be distilled, partly unchanged, in the highest vacuum obtainable. When decomposed by means of ozone, it yields geronic acid in a proportion which, on the assumption that the vitamin molecule contains a single  $\beta$ -ionone ring, indicates the vitamin *A* content to be about 50–80 per cent. of the whole preparation. About 5 per cent. of vitamin *D* is also present.

T. H. P.

## Agricultural.

**Neubauer's Chemico-Physiological Method for the Determination of Phosphoric Acid and Potash assimilable by Soil. I. C. Antoniani and M. Nicolini.** (*Giorn. Chim. Ind. Appl.*, 1931, 13, 369-372.)—According to this method (*Zeits. Pflanzenernähr.*, 1923), 100 corns of rye are treated with an anti-septic solution to destroy mould spores, and are then allowed to grow for 17 days at 18° to 20° C. in a mixture of 100 grms. of the fine soil with 50 grms. of quartz sand, the whole being moistened and covered with 250 grms. of the sand. During the growing period, the moisture lost by evaporation is made up by addition of water. After the lapse of 17 days, the whole of the rye plants are carefully freed from soil and moistened with 0.2 per cent. calcium acetate solution. The mass is then dried on a water-bath and calcined in a muffle furnace at 700-750° C. Determinations of the potassium and phosphoric acid are then made on the calcined material, and the results corrected by means of similar determinations on the original seeds. The amounts of potash and phosphorus pentoxide taken up by the plants are referred to 100 grms. of water-free soil. T. H. P.

## Organic Analysis.

**Determination of Starch in Sized and Finished Cotton Goods. R. G. Fargher and L. V. Lecomber.** (*J. Text. Inst.*, 1931, 22, 475-487.)—The method recommended is similar in general outline to that of Derrett-Smith (*ANALYST*, 1930, 55, 131), but differs therefrom in certain important details. A sample of the fabric (or yarn), weighing about 10 grms., is cut into squares of  $\frac{1}{8}$  inch side, and thoroughly mixed. The moisture content is determined on 1 grm., and at the same time 2.5 grms. are wetted out in a 100 c.c. conical flask with 60 c.c. of water, which is heated just to its boiling point, the neck of the flask being closed meanwhile with a combined glass pear bulb and stirring rod. When cold, the mixture is treated with 20 c.c. of 4 *N* sulphuric acid, and the flask is immersed in a rapidly-boiling water-bath for 2.5 hours, its contents being stirred vigorously during the first 3 minutes. The cooled mixture is then filtered through a 2-inch Buchner funnel, and the residual cotton is washed repeatedly with water (100 c.c. in all). To the filtrate, which is kept briskly stirred, 19 c.c. of 4 *N* sodium hydroxide solution is added slowly from a burette or pipette. The liquid is made up to 250 c.c., and its content of glucose determined by either of the two following methods:

(1) *Iodine method.*—Fifty c.c. are treated with 25 c.c. of 0.05 *N* iodine solution and, slowly and with constant shaking, with 3.12 c.c. more than the necessary amount of 0.5 *N* sodium hydroxide solution to give a neutral liquid (*cf.* Hinton and Macara, *ANALYST*, 1924, 49, 2). After standing for 10 minutes at 20° C., the solution is acidified by addition of 1.6 c.c. of 4 *N* sulphuric acid, and the excess of iodine is determined by titration, conveniently in a 250-c.c. glass-stoppered bottle, with 0.05 *N* sodium thiosulphate solution. If more than three-fifths of the iodine added has been used up by the glucose, the titration should be repeated on 25 c.c. of the solution, whilst, if the volume of iodine solution consumed is very small, a

more accurate result may be obtained by titrating 100 c.c. The necessary correction for the reducing substances produced from the cotton itself may be determined by treatment of the unsized yarn in the same way; alternatively, use may be made of mean correction values which have been determined. If 1 gm. of the dry sized sample uses up  $T$  c.c., and 1 gm. of the dry blank  $t$  c.c. of 0.05  $N$  iodine solution, the percentage starch content is given by  $0.417 \left[ T - \frac{t(100-c)}{100} \right]$ , where

$c$  is the percentage of total size; 1 c.c. of 0.05  $N$  iodine corresponds with 0.00405 gm. of pure starch or 0.00417 gm. of average commercial starch. For grey cotton and scoured cotton the mean values of  $t$  are 8.15 and 4.31 c.c., respectively.

(2) *Copper method*.—The solutions required are made by dissolving (A) 100 grms. of pure copper sulphate crystals to 1 litre and (B) 130 grms. of anhydrous sodium carbonate and 50 grms. of sodium bicarbonate to 1 litre. The volume of the glucose solution taken is 50 c.c. (25 c.c.) for yarn or cloth containing less than 2.5 per cent. (from 2.5 to 8 per cent.) of starch, or less, if still more starch is present. Fifty c.c. of the hydrolysate are heated to boiling point in a conical flask (about 120 c.c.). Nine grms. of a mixture of sodium bicarbonate (50 grms.) and sodium carbonate (130 grms.) are added to 45 c.c. of solution B, and 5 c.c. of solution A are added from a pipette. This mixture is heated to boiling point, poured rapidly into the boiling hydrolysate and well mixed in with a combined glass pear bulb and stirring rod. The flask is then kept for an hour immersed to the neck in an actively-boiling water bath. The precipitated cuprous oxide is collected in a sintered glass funnel containing a pad of finely shredded asbestos, and the analysis is completed as described by Clibbens and Geake (*J. Text. Inst.*, 1924, **15**, 127). If 1 gm. of the dry-sized material (blank) requires  $T$  ( $t$ ) c.c. of 0.04  $N$  permanganate, the percentage starch content is given by  $0.082 \left[ T - \frac{t(100-c)}{100} \right]$ . The mean value of  $t$  is 30.5 c.c. for grey yarns and 20.1 c.c. for scoured yarns.

Tables are given showing the starch equivalents, given by these two methods, for various commercial starches and flours, the starch equivalents of other substances which may be present in size mixings, etc. T. H. P.

#### Determination of "Uronic" Acids and Pentoses in Tanning Extracts.

H. Phillips. (*J. Inter. Soc. Leather Trades Chem.*, 1931, **15**, 465-479).—Uronic acids are determined by distillation over an oil-bath at about 130° C. about 0.5 gm. of the dry tanning extract with 100 c.c. of hydrochloric acid of sp. gr. 1.060, a slow current of air, free from carbon dioxide being drawn through the apparatus. The carbon dioxide evolved is absorbed in two wash bottles in series containing 0.1  $N$  baryta water. After the mixture has been gently boiled for about 4 hours the baryta solutions are allowed to settle, and 25 c.c. of the clear supernatant solutions are mixed and titrated with 0.1  $N$  hydrochloric acid. The weight of carbon dioxide found is multiplied by four and returned as uronic acids.

Pentoses are determined by distilling about 1 gm. of the material with 200 c.c. of hydrochloric acid (sp. gr. 1.065) saturated with sodium chloride, until

the distillate no longer colours aniline acetate ( $1\frac{1}{2}$  hours). One hundred c.c. of hydrochloric acid (sp. gr. 1.065) are added to the distillate, after which it is diluted to 500 c.c. To 200 c.c. of this are added 10 c.c. of 10 per cent. sodium bromide solution and 20 c.c. of 0.1 *N* sodium bromate solution. The stoppered bottle containing this mixture is left in a dark cupboard for 1 hour. Ten c.c. of 10 per cent. potassium iodide are then added, and the liberated iodine is titrated with 0.1 *N* sodium thiosulphate. One c.c. of 0.1 *N* sodium bromate is equivalent to 0.0024 grm. of furfural.

The presence of uronic acids and pentoses is indicative of the presence of encrusting substances derived from the cell-walls of plants, such as chestnut wood and oak wood.

R. F. I.

**Precipitation of Tannin by means of Hexamethylenetetramine.** A. T. Hough. (*J. Inter. Soc. Leather Trades Chem.*, 1931, 15, 406.)—The precipitation of tannin by hexamethylenetetramine has been known at least since 1913, but the author has made a study of the conditions. He found that there was most precipitation at pH 5.6, at 6.3, or over, there was little or no precipitation, and at pH values below 5.6 the precipitate became less, down to pH 4.3. On maintaining the pH value between 5.5 and 5.7 and varying the proportion of the reagents, it was found that the most favourable proportion was 17 c.c. of 4 per cent. hexamethylenetetramine to 8 c.c. of 4 per cent. solution of "tannin." If the concentration is over 0.5 grm. of tannin per litre, the precipitate is visible to the unaided eye; the sensitivity may, however, be greatly increased by adding to 10 c.c. of the test solution four drops of the following solution:—10 c.c. of saturated zinc acetate (30 per cent.), 10 c.c. of 30 per cent. ammonium acetate, 1 c.c. of glacial acetic acid and 10 c.c. of 30 per cent. hexamethylenetetramine. These constituents must be mixed in this order. The reaction is visible to the unaided eye in 2 or 3 minutes when the tannin is 1 part in 200,000. One part in a million can be detected by using the Tyndall beam.

R. F. I.

**Deterioration of Vegetable-tanned Leather on Storage.** R. F. Innes. (*J. Inter. Soc. Leather Trades Chem.*, 1931, 15, 480–494.)—Analyses of various leathers which had rotted to varying extents showed that the nitrogenous compounds of the leathers had been rendered partly soluble in water, that a considerable proportion of this soluble nitrogen was in the form of an ammonium salt, and only a small proportion in the form of amino acids. In the case of a leather which had become completely disintegrated, the amount of nitrogen soluble in water was 56 per cent. of the total nitrogen present. In less extreme cases this figure seldom exceeds 25 per cent. In partly rotted leathers the soluble nitrogen has been found to vary from 0.5 to 2 per cent. of the weight of leather, the ammoniacal nitrogen from 0.4 to 1.8, and the amino nitrogen from 0 to 0.14 per cent. This is considered to be proof that in the rotting of leather oxidation plays a more important part than acid hydrolysis. It is shown, however, that oxidation will only take place in the presence of acid, and if leather-bound books are stored in cities in such a way that they cannot absorb the sulphur compounds from the atmosphere, rotting does not take place.

Attempts to cause leather to rot by artificial means (e.g. by hydrogen peroxide and sulphuric acid) were successful in a number of cases, and soluble nitrogen was produced, but the ammoniacal nitrogen was less than in naturally rotted leathers. It was found that certain native-tanned Nigerian leathers resisted the action of peroxide and acid (*cf.* ANALYST, 1931, 762).  
R. F. I.

**Water Permeability of Leather.** R. S. Edwards. (*J. Inter. Soc. Leather Trades Chem.*, 1931, 15, 495-514.)—Water penetration tests on leather have, until recently, been too crude, and no allowance has been made for controlling (a) temperature, (b) the relative humidity of the air surrounding the "dry" side of the leather being tested. In the apparatus described the leather is held vertically between the rims of a pair of flanged cups tightly screwed on to seatings of rubber rings. Glass inspection windows are provided. Each flanged cup has an inlet and an outlet tube, so that water can be allowed to flow on to the one surface of the leather, and air of a definite degree of relative humidity (100, 66, 33, and 0 per cent.) on to the other surface. This is controlled by passing the air at the rate of 1.7 c.c. per second at N.T.P. through two wash bottles (in series) containing sulphuric acid of the requisite concentration. After this, the air stream is divided into two, one current passing through two wash bottles of sulphuric acid of the same concentration as the previous pair, and the other current passing across the surface of the dry leather in one of the flanged cups and then into two other wash bottles of sulphuric acid. The difference in the change of weight in these gives the effect of passing the air over the leather face. Temperature is controlled by placing the whole apparatus in an incubator.

There is no need for any special mechanism to denote the time of penetration. The lower the relative humidity of the air on the "dry" side and the higher the temperature, the greater the increase in time of penetration.  
R. F. I.

**Colour Reaction of Colophony and Abietic Acid.** H. Griffon. (*J. Pharm. Chim.*, 1931, 123, 337-339.)—Colophony and its essential constituent, abietic acid, give a very fleeting colour in the Liebermann reaction, but an intense blue-violet colour is given by very small proportions of colophony, and a blue colour by pure abietic acid, in Pettenkofer's reaction. The reaction may be applied directly to soaps by dissolving a few cgrms. in 95 per cent. alcohol, adding 10 drops of a 1 per cent. solution of furfural in alcohol, and pouring 2 c.c. of sulphuric acid down the side of the tube. In the presence of colophony a red colour is immediately seen at the junction of alcohol and acid, and rapidly turns violet-blue. Although commercial soaps do not generally contain sterols giving this reaction, it is advisable to carry out a Liebermann test with a solution of the soap in acetic anhydride.

D. G. H.

**Determination of *m*-Xylene in Xylene Mixtures.** H. P. Reichel. (*Chem. Ztg.*, 1931, 55, 744-745.)—Five c.c. of sample are pipetted at 15° C. into a 500 c.c. flask containing 20 c.c. of glacial acetic acid and cooled in ice-water; 25 c.c. of nitric acid (sp. gr. 1.5) are then added (the mixture being shaken and cooled well), followed by 40 c.c. of sulphuric acid (66° Bé). After 30 minutes at 20° C. and

1 to 2 hours on the water-bath, the flask is cooled in ice, 450 c.c. of water are added, the liquid is again cooled for 30 minutes, and the precipitate is filtered off by suction, the acid being washed out with 150 c.c. of water. It is then heated under a reflux condenser with 100 c.c. of acetone for 30 minutes, recrystallised in an ice-bath, filtered off at 15° C., dried for 1 hour at 95° C. and weighed. This residue should be pure 2,4,6-trinitro-*m*-xylene (m.pt. 182° C., solubility in acetone 0.6 gm. per 100 c.c. at 15° C.) free from 2,3,5-trinitro-*p*-xylene (solubility 5.0 grms. per 100 c.c., m. pt. 140° C.), 2,3-dinitro-*p*-xylene (solubility 30 grms. per 100 c.c.), and other nitro-derivatives (mainly liquid) produced under these conditions from *o*-xylene, ethyl benzene and other constituents of commercial xylene. If necessary, it should be recrystallised a second and third time from acetone under the above conditions, 0.6 gm. being added to the weight for every 100 c.c.-portion of acetone used. Then, corrected weight  $\times 10.2$  = percentage of *m*-xylene, the error for mixtures containing 20 to 60 per cent. in the presence of the above compounds and paraffin hydrocarbons not exceeding 2 per cent. J. G.

**Determination of some Derivatives of Phenol and of Salicylic Acid from the weight of Lautemann's Red.** M. François and L. Seguin. (*Bull. Soc. Chim.*, 1931, 49-50, 1222-1229.)—For ordinary phenol, 10 c.c. of dilute alkaline solution, containing about 0.2 gm., are heated on the water-bath in a 250 c.c. conical flask with 5 c.c. of pure sodium hydroxide solution (sp. gr. 1.33) and 100 c.c. of water for 20 minutes. A solution containing 12.70 grms. of iodine and 20 grms. of potassium iodide per 100 c.c. is then added until the solution is distinctly brown, and the heating is continued for a further hour, an excess of iodine being always ensured. "Lautemann's red" is deposited, and is separated by filtration on a double paper after removal of excess of iodine from the cool solution by addition of the necessary amount of neutral sodium sulphite solution. It is washed 8 times and dried at 100° C., when weight  $\times 0.2732$  = weight of phenol. The method may also be applied successfully to salicylic acid in sodium and bismuth salicylates and to aspirin, salol, betol and, possibly, to phenolphthalein, the sample being dissolved or saponified, according to its nature, in 100 c.c. of a hot solution containing 5 c.c. of the alkali solution. Methyl salicylate (0.3 gm.) is weighed into a tared 250 c.c. flask, 50 c.c. of water, 50 c.c. of 95° alcohol and 20 c.c. of the alkali solution are added, and the mixture is heated under a reflux condenser for 2 hours or until homogeneous. The alcohol is then removed on the water-bath, the residue extracted with water, the extract and washings being made up to 200 c.c., and the salicylic acid is determined on 20 c.c. Lautemann's red,  $C_{12}H_4I_4O_2$  (cf. Bougault, *J. Pharm. Chim.*, 1908, 28, 145) is an aristol, *i.e.* an insoluble, usually coloured, compound of phenol and iodine, but differs from the aristols of other (*e.g.* di- and tri-atomic) phenols in that it is produced more slowly. The authors have found it advantageous to substitute sodium hydroxide for sodium carbonate in the original method of preparation (*loc. cit.*). Since 1 molecule corresponds with 2 molecules of phenol or salicylic acid, the amount of substance to be determined (molecular weight  $M$ ) is given by the ratio  $M/344$ . J. G.

## Inorganic Analysis.

**Volumetric Benzidine Method for the Determination of Acetic Acid (Acetate Radicle) in Lead Acetate.** J. E. S. Han and T. L. Chu. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 379-381.)—The following method depends on precipitating the lead by sulphuric acid in presence of alcohol, removing the excess of sulphuric acid by precipitation as benzidine sulphate, and determining the acetic acid remaining in the solution by titration with sodium hydroxide. The sample of lead acetate (0.94 to 0.95 grm.) is dissolved in 20 c.c. of water, and 26 c.c. of dilute sulphuric acid (0.2 *N*) are added (both the water and the dilute sulphuric acid must be free from carbon dioxide). Fifty c.c. of neutralised dilute alcohol (prepared by neutralising 96 per cent. alcohol with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator, and diluting 90 c.c. of this with 81 c.c. of water) are added. The precipitate of lead sulphate is filtered off after 30 minutes and washed with 50 c.c. of the dilute alcohol, followed by 25 c.c. of water. To the filtrate, 5 c.c. of alcoholic benzidine solution (0.921 per cent.) are added; after 5 minutes the precipitate of benzidine sulphate is filtered off (9 cm. paper) and washed with 50 c.c. of water. The filtrate is titrated with 0.1 *N* sodium hydroxide (free from carbonate), 4 drops of alcoholic phenolphthalein (1 per cent.) being used as indicator. The results of analyses, by this method, of samples of lead acetate from different sources, and also of lead subacetate, show excellent agreement with those obtained by a method involving distillation with phosphoric acid, for the details of which the memoir should be consulted.

S. G. C.

**Determination of Mercury as Periodate.** H. H. Willard and J. J. Thompson. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 398-399.)—The mercury solution (150 c.c.) in dilute nitric acid (not stronger than 0.15 *N*) or in dilute sulphuric acid (not stronger than 0.1 *N*) is heated to boiling, and the mercury is precipitated as the orange-red periodate by adding slowly, with constant stirring, a solution of 2 grms. of sodium or potassium periodate in 50 c.c. of water. The cooled liquid is filtered through a crucible with a porous bottom; the precipitate is washed with warm water, dried at 100° C. for 2 to 3 hours, and weighed as  $\text{Hg}_5(\text{IO}_6)_2$ . Instead of weighing the precipitate, it can be dissolved and the mercury determined in the following manner:—The washed precipitate of mercuric periodate is dissolved in the crucible by adding 2 or 3 grms. of solid potassium iodide and 15 c.c. of water, and stirring. The solution, washed into a flask, is acidified with 10 c.c. of 2 *N* hydrochloric acid, and the liberated iodine is titrated with 0.1 *N* sodium thiosulphate solution, with starch as indicator. Iodine is liberated according to the equation



Good results were obtained in test experiments with 0.05 to 0.36 grm. of mercury alone, and in presence of about its own weight of nickel, aluminium, cadmium, zinc and copper. Chlorides interfere.

S. G. C.

**Electrolytic Determination of Cobalt.** D. H. Brophy. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 363-365.)—More or less unsatisfactory results were obtained in the electrolytic determination of cobalt from ammoniacal solution, as recommended by previous investigators. An improvement has been effected by depositing cobalt, from an electrolyte containing 50 c.c. of ammonia (strength not stated), 5 grms. of ammonium chloride and 0.3 gm. of sodium bisulphite in a volume of 100 c.c., upon a platinum-rhodium gauze cathode, employing a platinum anode rotating at 800 to 1000 revs. per minute, and a C.D. of 4 to 7 amps. per sq. dm.; the deposition takes half-an-hour. Good results were obtained with 12.5 to 162 mgrms. of cobalt. For amounts of cobalt below 100 mgrms. it is recommended to use 0.4 gm. of sodium bisulphite in the electrolyte, *i.e.* somewhat more than the normal quantity; apparently, also, it is not clear why more reducing agent is required for the smaller amounts of cobalt. The presence of bisulphite in the solution is essential, but the way in which it acts is not known. A more prolonged period of electrolysis leads to high results, owing to deposition of platinum dissolved from the anode. Cobalt must be separated from "any obviously interfering elements" before the electro-deposition. S. G. C.

**Volumetric Determination of Manganese after Oxidation by Periodate.** H. H. Willard and J. J. Thompson. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 399-401.)—Willard and Greathouse (*J. Amer. Chem. Soc.*, 1917, 39, 2366) employed potassium periodate to oxidise manganese to permanganate, and determined the permanganate colorimetrically. The present authors have extended this method by precipitating the excess of periodate as mercuric periodate, thus permitting the titration of the permanganate in the ordinary way.

**Manganese in steel (containing less than 0.1 per cent. of chromium).**—One gm. of the steel is dissolved in a mixture of 15 c.c. of water and 15 c.c. of phosphoric acid (85 per cent.). The solution is oxidised with 1 to 2 c.c. of nitric acid, boiled to remove nitrous fumes, and diluted to 100 c.c.; 0.3 gm. of sodium or potassium periodate (sufficient for up to 15 mgrms. of manganese) are added, the solution is boiled gently for 15 minutes to oxidise the manganese, diluted to 150 c.c., and cooled to room temperature. The periodate is precipitated by adding slowly, with constant stirring, a strong solution of 2 to 3 grms. of mercuric nitrate, the liquid is filtered immediately through an asbestos filter into a measured excess of standard ferrous sulphate solution containing 10 c.c. of sulphuric acid (50 per cent.), and the precipitate is washed with water. The excess of ferrous sulphate is titrated with standard permanganate solution.

**Manganese in iron (oxide) ore.**—One gm. is heated with 15 c.c. of phosphoric acid (85 per cent.) and a few drops of sulphuric acid until fumes of sulphuric acid appear. The cooled residue is dissolved in water (100 c.c.); the rest of the process is the same as for steel.

**Manganese in bronze.**—One gm. is dissolved in a mixture of 3 c.c. of concentrated nitric acid, 10 c.c. of phosphoric acid (85 per cent.), and 8 c.c. of water. The liquid is diluted to 50 to 75 c.c., and the process is continued as for steel.



Owing to the deep blue colour of the solution, it is recommended to carry out the final titration with permanganate electrometrically or by the use of alphazurine indicator (*cf.* Knop, *Z. anal. Chem.*, 1929, 77, 125). S. G. C.

**Iodimetric Determination of Chromium.** E. Schulek and A. Dózsa. (*Z. anal. Chem.*, 1931, 86, 81-92.)—The cold solution of chromic sulphate (20 c.c.) is treated with 2 to 5 c.c. of fresh (*i.e.* bromate-free) saturated bromine water, followed by 10 c.c. of 30 per cent. sodium hydroxide solution, and heated only to incipient boiling. After thorough cooling, 5 c.c. of 5 per cent. phenol solution are added all at once, which destroys the excess hypobromite. The solution is next transferred to a conical flask provided with a well-fitting glass stopper, and diluted to 400 c.c.; 4 grms. of potassium bicarbonate are dissolved in the liquid, which is then treated with 50 per cent. sulphuric acid in small portions until its yellowish colour changes to reddish, when potassium iodide (2 grms.) is added. This is followed by more of the sulphuric acid (25 c.c. in all); the flask is then stoppered at once. The iodide is made to react near the neutral point, to prevent any reaction of bromide upon chromate in the more acid medium. After 5 minutes' standing, the solution is titrated with thiosulphate solution; 1 c.c. of 1 *N* = 1.733 mgrm. Cr. For a separation from *iron*, the sodium hydroxide precipitate must be dissolved and re-treated, the filtrates being combined; if the chromium is high, a third precipitation should be made. If *nickel* is present, the combined alkaline filtrates should be acidified with 15 c.c. of sulphuric acid and boiled so that any bromate—formed by induced autoxidation of hypobromite in contact with nickelic oxide—may be decomposed. During this boiling, a small fraction of the chromate is reduced by bromide; hence the cooled solution is oxidised once more with 1 to 2 c.c. of bromine water, caustic soda, etc., with phenol treatment as above. The method should not be used for nickel and chromium-bearing materials high in chromium. W. R. S.

**Determination of Gallium.** A. Brukl. (*Z. anal. Chem.*, 1931, 86, 92-94.)—A slight loss in gallium, which has been found to occur when precipitates containing gallium together with organic matter are ignited, has been traced to the formation of the volatile monoxide  $Ga_2O$ . This has been prepared by the reduction of the sesquioxide in hydrogen; it begins to sublime at 660° C. at ordinary pressure. The cupferron and tannin precipitates of gallium (*ANALYST*, 1929, 54, 64, 267; 1930, 55, 218) should, therefore, be treated by wet methods for the destruction of the organic matter, and the hydrated sesquioxide precipitated with ammonia. The precipitate is dissolved in nitric acid, the solution is evaporated in a tared platinum crucible, and the residue is ignited to  $Ga_2O_3$  in an electric furnace. The oxide attacks quartz and porcelain at ignition temperatures, and may be partly reduced by diffusing burner gases if the platinum crucible is heated in a gas flame. W. R. S.

**Quantitative Determination of Calcium by means of Picrolonic Acid.** R. Dworzak and W. Reich-Rohrwig. (*Z. anal. Chem.*, 1931, 86, 98-113.)—Picrolonic acid (1, *p*-nitrophenyl-3, methyl-4, nitro-pyrazolon 5) gives with solutions

of calcium salts a voluminous, yellowish-green precipitate which changes to a dense, crystalline pale-orange powder, of the composition  $\text{Ca}(\text{C}_{10}\text{H}_7\text{N}_4\text{O}_5)_2 \cdot 8\text{H}_2\text{O}$  (Ca factor, 0.05641). The reagent is a 0.01 *N* aqueous filtered solution of the acid, prepared by warming and stirring (2.64 grms. per litre). Its strength may be ascertained by titration with alkali against phenolphthalein. The calcium solution, neutralised to litmus, containing not more than about 0.1 gm. of metal, and not more than 1 gm. of magnesium, alkali, and ammonium salts, is stirred and titrated with the reagent until a precipitate has formed; if it is flocculent, the liquid is warmed and stirred until the precipitate becomes crystalline. Further addition of reagent is regulated by the speed of transformation of the flocculent into the crystalline form, heating and stirring being resorted to if necessary. When the precipitation is complete, an excess of reagent (more than half the volume required for precipitation) is added. After standing several hours, the precipitate is collected in a porous crucible, washed with cold water, dried at ordinary temperature in a current of dust-free air, or by simple standing, and weighed. The salt dried by heating is hygroscopic and contains between 7 and 8 molecules of water. The process is very suitable for micro-work. It provides a quantitative separation of calcium from magnesium, unless the proportion of the latter exceeds 10 times the quantity of the former. For the determination of lime in natural waters, 100 c.c. are treated with the same bulk of reagent. The precipitate forms in the crystalline condition; after some hours it is collected and treated as described.

W. R. S.

**Improved Method for the Determination of Magnesium by means of *o*-Hydroxyquinoline.** F. L. Hahn. (*Z. anal. Chem.*, 1931, 86, 153-157.)—The reagent is a solution of 38 grms. of the base in 125 to 150 c.c. of 2 *N* hydrochloric acid, made up to one litre. One c.c. precipitates approximately 0.003 gm. of magnesium. The solution (about 20 c.c.) containing 0.005 to 0.1 gm. of metal and contained in a 250 c.c. measuring flask, is treated with 2 to 3 grms. of ammonium chloride, 2 to 3 c.c. of strong ammonia and 20 c.c. of acetone, heated to water-bath temperature, and titrated gradually with small amounts of the reagent. The precipitate may not form at once; it is very coarse and settles quickly, and hence the colour of the supernatant liquid can easily be observed. The addition of reagent is continued until the hot liquor is as yellow as the precipitate. If much reagent is required, more acetone is advisable (as a solvent for the base). The coloured liquid is diluted with 10 c.c. of acetone, and water to about 200 c.c., stirred, and left for some hours or overnight. It is then made up to the 250 c.c. mark, mixed and filtered, and 100 c.c. are transferred to a porcelain basin. Acetic acid is added until phenolphthalein is decolorised, followed by 5 c.c. of 5 per cent. zinc sulphate solution, which precipitates the excess of base and thus prevents its volatilisation. The solution is heated on the water-bath for half-an-hour to ensure removal of the acetone, and transferred to a conical flask, and the basin is washed with 30 c.c. of strong hydrochloric acid, which is added to the liquid in the flask. This is treated with a known excess of bromate-bromide solution (28 grms.  $\text{KBrO}_3$  and

50 grms. KBr per 1000 c.c.), and the excess of bromine is titrated with iodine thiosulphate (ANALYST, 1927, 52, 431). For smaller quantities of magnesium (up to 0.01 gm.), 10 c.c. of solution are treated in a 100 c.c. measuring flask, and the quantities of the reagents reduced proportionately.

W. R. S.

**Determination of Rubidium and Caesium, particularly in Mineral Waters.** L. Fresenius. (*Z. anal. Chem.*, 1931, 86, 182-190.)—The gravimetric method is based on some recent investigations (ANALYST, 1926, 51, 162; 1928, 53, 459; 1930, 55, 465), combined with an improved process for the elimination of the bulk of the potassium by repeated precipitation with alcoholic hydrochloric acid. An amount of mineral water furnishing 500 to 700 grms. of mixed alkali chlorides is concentrated in a porcelain basin to about 4 litres and boiled with sodium hydroxide and carbonate, the alkaline-earth precipitate being filtered off and washed with dilute sodium carbonate solution. The filtrate is acidified with hydrochloric acid and treated at 80° C. with cobalt nitrate and sodium nitrite. The precipitate is stirred at intervals for a day; after addition of 50 c.c. of acetic acid and another day's standing, it is collected and washed 3 to 4 times with 10 per cent. acetic acid. It contains all the potassium, rubidium, and caesium with a little sodium; it is digested for an hour at 70° C. with ammonia and ammonium sulphide, and the cobalt precipitate is filtered off and washed with dilute ammonium sulphide, and tested for alkali metal. The filtrate is evaporated to dryness with a large excess of hydrochloric acid; this treatment is repeated twice, the last residue being dissolved in very dilute acid and the sulphur filtered off. The boiling filtrate is treated with hot barium chloride solution and filtered, and the excess of barium in the filtrate is precipitated with ammonium carbonate. After another filtration, the liquid is evaporated to dryness, and the residue is gently ignited until the ammonium salts are expelled.

**Separation of potassium, rubidium, and caesium.**—The residual chlorides are weighed and dissolved in four times their weight of water; the solution is treated with 2.5 times its volume of a mixture of fuming hydrochloric acid and 96 per cent. alcohol (1:2). The precipitate (potassium chloride) is collected after 2 hours, washed 3 times with the alcoholic acid, and treated twice more by the same process. The combined filtrates and washings are evaporated to dryness on a water-bath, the residue is weighed, dissolved in 4 parts of water, and the precipitation with the alcoholic acid repeated three times as before. These threefold extractions with alcoholic acid are repeated until the concentrated liquor no longer yields a precipitate of potassium chloride. The solution is then evaporated to dryness, and the residue is dissolved with an equal amount of ferric chloride in a minimum of water; after addition of 50 c.c. of glacial acetic acid per gm. of mixed chlorides, the solution is heated almost to boiling and precipitated with one c.c. of a 40 per cent. solution of antimony trichloride in glacial acetic acid. The precipitate (caesium chloro-antimonite) is left on the water-bath for one hour, and for 12 hours at ordinary temperature. It is collected in a porous-glass crucible, washed with a 5 per cent. solution of antimony chloride in glacial acid, and dissolved in hot water

containing hydrochloric acid. The solution is treated with hydrogen sulphide and filtered, and the filtrate is evaporated with perchloric acid until heavy fumes are given off. When cold, the acid is diluted and stirred with 5 c.c. of 0.3 per cent. alcoholic solution of perchloric acid; after 1 hour, the precipitate is collected in a porous crucible and washed with alcohol, dried at  $130^{\circ}\text{C}.$ , and weighed as  $\text{CsClO}_4$ , which is tested spectroscopically for purity (especially for potassium and rubidium).

The filtrate from the caesium chloroantimonite is precipitated with hydrogen sulphide, the antimony sulphide is filtered off, and the filtrate is evaporated to dryness. The residue is dissolved in four parts of water, and the solution is diluted with  $2\frac{1}{2}$  parts of alcoholic hydrochloric acid (*supra*) and precipitated with 1 c.c. of a boiling 40 per cent. solution of stannic chloride in absolute alcohol. The rubidium chlorostannate is collected after 12 hours in a porous crucible, washed with absolute alcohol, and dried at  $110^{\circ}\text{C}.$  to constant weight. Alternatively, it may be converted into, and weighed as, perchlorate in the same manner as the caesium chloroantimonite. The weighed rubidium compound is tested spectroscopically for purity (potassium and caesium).

W. R. S.

#### Determination of Fluorine in Phosphate Rock and Phosphate Slag.

D. S. Reynolds and K. D. Jacob. (*Ind. Eng. Chem., Anal. Ed.*, 1931, 3, 366-370.)

—The fluorine in highly phosphatic, calcareous materials, such as phosphate rock, cannot be brought into solution by a single fusion with an alkaline flux. Three fusions usually fail to convert more than about 90 per cent. of the fluorine into a soluble form. It has been found that during the extraction of the melt with water, fluoride ions react with the other products of the fusion to form insoluble fluorophosphates of the fluorapatite type. By extraction of this insoluble residue with dilute nitric acid, however, the fluorine which it contains can be brought into solution, together with calcium and phosphoric acid. The method described below for the analysis involves (a) obtaining the fluorine in solution, (b) separating the silica, etc., (c) determining the fluorine volumetrically by means of the lead chlorofluoride method (the latter two parts of the process are an adaptation of Lundell and Hoffman's method—*Bureau of Stds. J. Research*, 1929, 3, 581). One gram. of the finely powdered phosphate rock (100 mesh), 0.5 gram. of silica, and 2 grms. of sodium carbonate, are fused in platinum at  $900^{\circ}$ – $950^{\circ}\text{C}.$  for 1 hour (in the case of phosphate slags, 1 gram. is fused with 5 grms. of sodium carbonate without added silica). The cooled melt is digested overnight on a steam-bath with 50 to 75 c.c. of water, and the liquid is decanted on to a filter. The residue is broken up, digested with 50 c.c. of dilute sodium carbonate solution (1 per cent.) on a steam-bath for 15 minutes, the residue is filtered off on the filter previously used, washed several times with hot water, and the combined filtrates are evaporated to about 50 c.c. volume (solution A, reserved). The residue is washed back into the digestion vessel with about 50 c.c. of water; 3 c.c. of concentrated nitric acid are added; the mixture is kept for 1 hour, with frequent stirring (solution B); any residue is filtered off, fused with sodium carbonate and the extract of this fusion is added to solution A. Fifty c.c. of dilute oxalic acid solution (5 per cent.) are

added to solution B, and the calcium oxalate is precipitated by adding dilute sodium carbonate solution (10 per cent.), drop by drop, until the liquid is neutral to methyl orange indicator. The liquid is boiled for 1 minute, cooled, and filtered, and the precipitate is washed 4 or 5 times with cold water; the precipitate is rejected. The filtrate is acidified with an excess of 4 c.c. of nitric acid, 10 c.c. of saturated potassium permanganate are added; the liquid is warmed, and when the permanganate colour has disappeared, more permanganate solution is added, drop by drop, until the liquid is permanently coloured or a brown precipitate forms. The excess of acid is then neutralised by adding solid sodium carbonate (until frothing ceases), followed by 2 grms. in excess (if the precipitate is light coloured, permanganate solution is added until it becomes dark brown); the liquid is boiled and filtered, and the precipitate is washed with hot sodium carbonate solution (1 per cent.). The filtrate is united with solution A, and the total volume is adjusted to 250 c.c.

*Separation of silica, etc.*—This solution is heated to boiling, and 25 c.c. of zinc nitrate solution [(5 grms. of zinc oxide dissolved in 100 c.c. of dilute nitric acid (1:9)] are added, with continuous stirring. The liquid is filtered through a rapid-filtering 15 cm. paper; the precipitate (containing a part of the silica) is washed and discarded. The filtrate (400 c.c.) is neutralised to methyl red indicator with nitric acid, 25 c.c. of ammoniacal zinc oxide solution [5 grms. of zinc oxide dissolved in warm water containing 10 per cent. of ammonium carbonate and 10 per cent. of ammonia (sp. gr. 0.90)] are added, and the liquid is evaporated to a volume of 50 c.c. The precipitate (containing the rest of the silica) is filtered off, washed with cold water and discarded.

*Determination of the fluorine.*—To the filtrate (250 c.c.) 2 drops of bromophenol blue indicator are added; it is rendered slightly acid with nitric acid and then just alkaline with sodium hydroxide, and 3 c.c. of sodium chloride solution (10 per cent.) and 2 c.c. of dilute hydrochloric acid (1:1) are added, followed by 5 grms. of solid lead nitrate. The liquid is heated on a steam-bath to dissolve the lead nitrate, 5 grms. of sodium acetate are added, and the solution is heated for 30 minutes and kept overnight. The precipitate of lead chlorofluoride is filtered off, washed once with cold water, five times with a saturated solution of lead chlorofluoride, and finally once with water. The precipitate is dissolved in 100 c.c. of warm dilute nitric acid (1:19); a slight excess of 0.1 N silver nitrate solution is added (a total of 20 c.c. is usually sufficient); the liquid is heated on a steam-bath for 30 minutes, cooled and filtered, and the precipitate of silver chloride is washed with cold water. The excess of silver in the solution is titrated with 0.1 N potassium thiocyanate solution, whence the amount of silver required to combine with the chlorine in the lead chlorofluoride is obtained; 1 c.c. of 0.1 N silver nitrate solution = 0.00190 grm. of fluorine.

S. G. C.

**Colorimetric Method for the Determination of Carbon Dioxide.** E. M. Emmert. (*J. Assoc. Off. Agr. Chem.*, 1931, 14, 386-389).—When carbon dioxide is shaken with a solution of the sodium salt of phenolphthalein the red colour

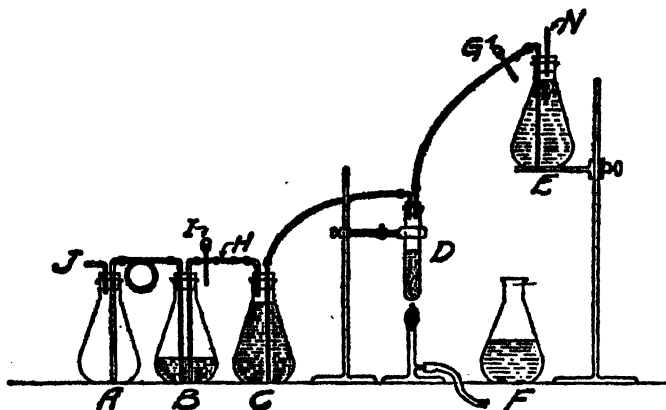
diminishes in intensity in proportion to the amount of carbon dioxide present, owing to the formation of sodium bicarbonate, which removes the sodium from the coloured salt and leaves the phenolphthalein colourless. In using this reaction to determine carbon dioxide, entry of other acidic or basic substances must be prevented by bubbling the gas through 1 per cent. sulphuric acid (10 c.c. of the concentrated acid diluted to 1 litre). The following reagents are used: (1) Neutral alcohol, prepared by adding, to 95 per cent. alcohol, a few drops of 0.5 per cent. phenolphthalein solution and then *N* sodium hydroxide solution dropwise until a faint pink colour persists. (2) Solutions of the coloured sodium salt of phenolphthalein. In each case, 100 c.c. is used, and the strength varies with the amount of carbon dioxide to be determined, as follows:

Amount of carbon dioxide. Mgrms.	Concentration of the sodium salt.
2 to 9	0.0025 <i>N</i>
9 to 18	0.0050 <i>N</i>
18 to 35	0.0100 <i>N</i>
35 to 50	0.0150 <i>N</i>
50 to 70	0.0200 <i>N</i>
70 to 90	0.0250 <i>N</i>
90 to 120	0.0300 <i>N</i>

Slightly more than the amount of phenolphthalein required for 2 litres of solution is dissolved in the neutral 95 per cent. alcohol, made up to 1 litre with the alcohol, and mixed with 1 litre of aqueous solution of the required amount of sodium hydroxide. For accurate work the solutions thus made, which must be kept from the air, should be standardised either by titrating against standard acid or by using the sodium carbonate solution (3) and following the procedure described below. (3) 12.05 grms. of pure anhydrous sodium carbonate are dissolved in 200 c.c. of water and made up to 500 c.c.; 1 c.c. = 10 mgrms. of  $\text{CO}_2$ . (4) 50 per cent. sulphuric acid (500 c.c. of the acid + 500 c.c. of water).

The liquid or solid, containing 5 to 120 mgrms. of carbon dioxide, is placed in the test tube D (this should be large for large samples), which is filled to within 10 to 15 c.c. with water. One hundred c.c. of the appropriate solution (2) are placed in flask B, which is filled to within 10 to 20 c.c. with 1 per cent. sulphuric acid. F contains reagent (4) and stopcock I should be open. When all connections are made air-tight, the stopper and tubes from E are transferred to F and 10 to 15 c.c. of the reagent (4) forced into D by blowing at N. The cock G is then closed and the stopper and tubes are replaced in E without destroying the siphon formed. If much  $\text{CO}_2$  is present, the acid should be blown over slowly. The liquid in D is boiled for several seconds, but not boiled over into C to any extent. The flame is then withdrawn, G is at once opened, and the gases forced from D and C by the 1 per cent. sulphuric acid from E until the liquid reaches the glass tube H (4 to 5 inches long), the clip I being then closed tightly and no acid solution allowed to enter B. The connection at H and the other tubes leading to B should be washed out after each determination. Flask B is disconnected from C at H and is lowered

below A and shaken vigorously until its contents show no further change in colour. If the solution in B becomes colourless or nearly so and much coloured solution has been forced into A, most of the liquid from B is drawn into A by applying suction at J. The suction is then released and the vacuum in B allowed to draw back all the solution it can. If B contains but little solution, this will probably be decolorised several times, repetition of the mixing by suction being thus rendered necessary. If the liquids in both A and B become nearly or quite colourless either the sample contained too much carbon dioxide or acid has passed into B.



APPARATUS FOR COLLECTING  $\text{CO}_2$  OVER THE SODIUM SALT OF PHENOLPHTHALEIN

When change in colour intensity has ceased, the tubes are removed and the solutions in A and B mixed and compared, as regards colour, with the particular reagent used. The quantity (mgrms.) of  $\text{CO}_2$  in the sample taken is given by  $\frac{Y - YR}{U}$ , where Y is the theoretical or determined number of mgrms. of carbon

dioxide equivalent to 100 c.c. of the reagent (2) used, R the original colorimetric reading of the reagent (2), and U the colorimetric reading of the reagent after being shaken with the carbon dioxide. The carbon dioxide of the air enclosed in the shaking flasks introduces an error of 0.1 to 0.2 mgrm., which is of importance when from 2 to 5 mgrms. is to be determined.

The carbon dioxide in a gas may be determined by introducing a known volume of the gas (at definite temperature and pressure) over the reagent (2) by displacement. If the amount of carbon dioxide in the gas is small, it should first be absorbed in concentrated sodium hydroxide solution.

T. H. P.

## Reviews.

A COMPREHENSIVE TREATISE ON INORGANIC AND THEORETICAL CHEMISTRY. Vol. XI. By J. W. MELLOR, D.Sc., F.R.S. Pp. xii + 909. London: Longmans, Green & Co., Ltd. 1931. Price 63s. net.

The elements described in this volume are tellurium, chromium, molybdenum, and tungsten. Of these metals, the chemistry of the last three is very far from

being satisfactorily understood, despite the facts that they have undergone considerable investigation and constitute the subject-matter of a very large number of original memoirs. There are, however, indications that the researches now being carried out with the aid of modern physico-chemical methods are helping to shed light on some of the many problems connected with their chemistry.

In preparing this volume Dr. Mellor has been confronted with the almost impossible task of forcing order into a branch of chemistry, where, in reality, very little order prevails. In so doing, he has evidently worked on the false assumption that many of the so-called compounds which have been described are indeed *true compounds* and not *chance mixtures*. Moreover, he has given them unfamiliar names, in accordance with his system of nomenclature. Nevertheless, it must be conceded that, in all probability, Dr. Mellor has provided us with as good a *résumé* of these elements as is possible by any living compiler. Volume XI is eminently worthy to take its place beside its excellent predecessors. In it, the references appear to be up-to-date, though a few instances occur where important papers published during the last two years have escaped the author's exceptionally vigilant eye.

A few loose statements have been noticed. Thus, on page 86, it is stated that "if telluric acid ionises in accord with  $\text{H}_6\text{TeO}_6 = \text{H}^+ + \text{H}_5\text{TeO}_6^-$ ," (which, incidentally, has recently been proved not to be the case), its ionisation constant is  $1.6 \times 10^{-4}$ , and it, therefore, "is a very weak acid approximating boric and hydrocyanic acids." Actually, such an acid would be about 100,000 times as strong as boric acid, and a million times as strong as hydrocyanic acid. It happens that telluric acid in solution behaves as a normal dibasic acid, and in its first stage ionises to an extent slightly greater than that of boric acid, and in its second stage a little less than hydrocyanic acid. On page 221 the work of Margaillan on chromic acid is referred to as involving "the e.m.f. of hydrogenised platinum and the solution against a mercury cathode." Such a phrase is almost meaningless. The use of the words "anode" and "cathode" in reference to cells in the state of equilibrium in this volume is distinctly unsatisfactory. This is so in the foregoing statement, and again on page 86, where the author refers to some work on the effect of telluric acid on the silver anode. It is hardly necessary, however, to add that these criticisms fade into insignificance when the vast wealth of information contained in the volume is considered.

In conclusion, Dr. Mellor is to be heartily thanked and congratulated on bringing his vast undertaking yet another stage nearer to its completion.

H. T. S. BRITTON.

RECENT ADVANCES IN ANALYTICAL CHEMISTRY. Vol. II, INORGANIC. Edited by C. AINSWORTH MITCHELL, D.Sc., F.I.C. Pp. xiv+452. London: J. & A. Churchill. 1931. Price 15s.

The title of this volume does not give a proper impression of the contents. While most of the book is devoted to a statement of recent advances in inorganic analytical chemistry, important sections are devoted to  $p\text{H}$  determinations,



microchemical methods, and water and sewage analysis. These three sections are as important as any others in the volume.

Chapter XVI, by Mr. J. W. Haigh Johnson, includes an informed and over discussion of the question of estimating pollution and of the attempts to determine it. The article gives a complete description of the methods described. The implied stricture in the footnote on page 389 is not deserved, for the issue of the booklet referred to does, to some extent, meet the situation described by Mr. Johnson on the same page, *viz.* "Difficult as this problem is, it is essential that uniformity should be secured." As matters are at present, the methods described are a good enough compromise for the time being, despite their known failings. A good deal more investigation will be required before an entirely satisfactory method of determining degree of pollution by chemical or biological tests is evolved. The nature of the difficulties to be overcome is clearly indicated here.

Very welcome sections of this chapter are the account of organisms as an index to pollution, and the significance of their absence.

Mr. Norman Evers contributes a short chapter on hydrogen ion concentration and potentiometric titrations. Some of the methods described are scarcely to be considered recent advances (see p. 9), and the description of the glass electrode is rather short. An account of the use of the electrometer valve, now available commercially, in conjunction with glass electrodes, would have been useful in view of its convenience and accuracy, for with the newer type of electrometer valve the wall of the glass electrode need not be quite so thin as was previously necessary. It would have been an advantage to have had an adequate account in this book of the recent application of potentiometric methods to non-aqueous solutions by the use of lithium chloride in place of potassium chloride, had these methods been available when the article was written.

The chapters dealing with the determination of lead, mercury, copper, cadmium, bismuth and other inorganic elements are full—so full in some cases as to become catalogue-like (see pages 28 and 116). It may be pointed out here that the use of tetramethyl-diamido-diphenylmethane for determining lead peroxide is older than is implied on page 22, and was extensively employed by Trillat for estimating traces of lead. Arsenic determinations receive full discussion, and the newer methods are adequately described by Dr. Evans, including his own work on the conditions for the separation of the element from other elements, published in *THE ANALYST*.

From time to time, workers are called upon to undertake an examination such as that of tellurium or selenium compounds, and this may involve much search through the literature, with the attendant difficulty of deciding on the suitable method. Dr. Schoeller's contribution on selenium and tellurium, therefore, fills a gap. It is full, and will enable the worker to select the process best suited to his problem.

The use of the newer reagents, such as 8-hydroxyquinoline, for the separation of aluminium from beryllium, is properly dealt with at length, as also is the application of that reagent to the determination of magnesium. The sensitive blue

lake, formed by magnesium with *p*-nitrobenzene-azo-resorcinol, is described, but not stressed, as it deserves to be, in its possible quantitative aspects.

Several sections are given up to the determination of constituents of iron alloys, and a few pages are assigned to gases in steel—a difficult field of analytical procedure, but one of increasing importance in the critical examination of steel.

The Editor contributes a short chapter on graphite and carbon blacks, wherein the requirements of commercial products are considered in some detail. The only comment on this section is that it might well have been extended.

The names of Powell and Schoeller at the head of sections on rare earth oxides and acids are a guarantee of thoroughness. Nor is the reader disappointed in the present instance. Mr. Powell collects the most recent information on the analytical behaviour of scandium, gallium and indium, and Dr. Schoeller deals with the niobium and tantalum separation. Those of us who have had to face the older methods for this separation appreciate the advances so far made by Dr. Schoeller and his co-workers in the tannin method of separation of the earth acids. These mark a definite step forward in this difficult field of analytical investigation.

Dr. Clarke and Mr. Etheridge contribute an excellent discussion on cobalt, nickel, manganese and zinc, which is the most complete compilation available of modern methods for determining these elements.

Dr. Janet W. Brown has compiled a readable chapter on Microchemistry, which may be regarded as a rather full summary of microchemical analysis. Fairly full accounts of the various types of balance are included, but the use of a good ordinary chemical balance by the method described by Conrady (*Proc. Roy. Soc.*, 1922, [A], 101, 211) is not dealt with. This method of weighing takes more time than that required by, say, a Kuhlmann balance, and cannot be pushed quite so far, but it utilises an ordinary balance and has the advantage of permitting the use of far greater loads. It is, therefore, available for accurate weighing of a few milligrams, and is entitled to be included as a microchemical technique. On page 319 we have the rather surprising statement that silver chloride is unsuitable for nephelometric measurement. It must be confessed that, in view of Richard's work and that of Wells and others, including our own experience, this dictum is rather startling. The inorganic section of this chapter is very good, but necessarily restricted in descriptive matter. It would, for example, have been an advantage if some of the neatly designed pieces of apparatus described by Feigl for microchemical tests could have been included. The use of coloured and impregnated threads rightly receives a couple of pages of lucid explanation, and there is a good table of reactions (spot and others) for elements. The quantitative micro-methods are more fully described, and include recent developments in apparatus and method.

The volume has a good index, and the bibliography at the end of each section is full. It is printed in bold type and is free from errors, as we have found by verifying the references in many cases. The contributors have done well, with the result that chemical laboratories must find a place for this and the previous volume. It is to be hoped that supplementary volumes will become available periodically to indicate further progress in analytical methods.

J. J. Fox.

**DIE MASSANALYSE. Zweiter Teil: DIE PRAXIS DER MASSANALYSE. 2<sup>e</sup> Auflage. (THE PRACTICE OF VOLUMETRIC ANALYSIS. Second Ed By I. M. KOLTHOFF, with the co-operation of H. MENZEL. Pp. Berlin: Julius Springer. Price, 28RM. (unbound); 29.40RM. (bound)**

This second German edition of Kolthoff's *Massanalyse* is no mere reprint of 1928 edition (for review, see ANALYST, 1929, 54, 257; English translation, see *ibid.*, p. 292); every chapter bears the mark of having been brought up to date. There has been no radical change in the contents and style of the book, but a new chapter on the use of ceric sulphate in volumetric analysis has been added. The present volume contains 100 pages more than the previous edition; the chapter on Practical Methods of Iodimetry has been extended by 30 pages, the new chapter occupies 9 pages, the index an additional 13 pages, and the remaining 48 extra pages are divided more or less equally among the remaining sixteen chapters. These figures indicate, to some extent, the changes in the new edition.

The present volume maintains the standard set in the first edition, and, although Kolthoff does not claim that the work is exhaustive, there seem to be surprisingly few satisfactory volumetric methods which do not find some mention. One of the reviewer's students had occasion recently to require a method for the analysis of mixtures of thionic acids, but none of the standard text-books consulted provided any useful information on the subject. A careful search of the literature revealed the work of Kurtenacker, but if this book by Kolthoff had come to hand somewhat earlier, the arduous search would have been avoided. This is only one example of the many topics previously to be found only in this book and in the original literature. Altogether, the work is one which can be warmly recommended.

S. GLASSTONE.

**ORGANIC SYNTHESSES, AN ANNUAL PUBLICATION OF SATISFACTORY METHODS FOR THE PREPARATION OF ORGANIC CHEMICALS. Editor-in-Chief: CARL S. MARVEL. Vol. XI, pp. iv+106. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1931. Price 8s. 6d. net.**

During the last ten years "Organic Syntheses" has become a recognised guide in practical organic chemistry, simply owing to the fact that the methods described in it are really reliable and give satisfactory results. To Vol. XI the usual annual welcome is, therefore, extended. Such names as W. Lloyd Evans, Louis F. Fieser, Erwin Ott, H. Staudinger, and E. J. Witzemann, among the contributors, and Roger Adams, H. T. Clarke, Henry Gilman and F. C. Whitmore among those who have checked the thirty-one preparations described, are in themselves the best guarantee of excellence the book can have. One feels certain that its users will again not be disappointed.

M. NIERENSTEIN

**A BRIEF INTRODUCTION TO THE USE OF BEILSTEIN'S HANDBUCH DER ORGANISCHEN CHEMIE. By E. H. HUNTRESS, Ph.D., Assistant Professor of Organic Chemistry, Massachusetts Institute of Technology. Pp. viii+35. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1930. Price 5s. net.**

The new Beilstein, or, as Prof. Huntress aptly calls it, the organic chemist's

Bible, is growing with alarming rapidity, and those of us who have been brought up on the old Beilstein approach it with awe. It is, therefore, a pleasure to be provided with a key to this treasure. No organic chemist could do better than to invest five shillings and get Professor Huntress's little book, which most lucidly explains the Prager-Jacobson system, the basis of the new Beilstein, and which provides numerous examples as a guide through this maze of organic-chemical data.

M. NIERENSTEIN.

**CHEMISTRY IN THE SERVICE OF MAN.** By ALEXANDER FINDLAY, D.Sc. Fourth Edition. Pp. xvi+355, with 40 illustrations. London: Longmans, Green & Co., Ltd. 1931. Price 6s. net.

The author has had the task of writing a volume on pure and applied chemistry that shall be readily intelligible to the general reader. This is a difficult task, because on the one hand he must remember all the time not to assume in his reader the background of scientific training that is so fundamental a part of his own outlook; on the other, he must not alienate his reader's sympathy by making him too conscious of his deficiencies in this direction, or by using too much space in an effort to remedy them, for the general reader will inevitably be more interested in the applications of chemistry in daily life than in tables of elements and chemical theories.

The appearance of this book in a fourth edition is in itself a very welcome sign that the author has, in some measure, succeeded in his task. It is obviously intended for the cultured reader who is prepared to take a serious interest in things chemical.

Quite early in the book the author plunges boldly into such deep waters as radioactivity and atomic structure; later, velocity of reaction and stereochemistry are touched upon. In places the argument is—perhaps of necessity—somewhat involved. As a rule, Professor Findlay is careful to expound the meaning of terms likely to be unfamiliar, but there are occasional slips. For example, the concept of heterogeneous reactions, introduced without adequate explanation, is calculated to mystify even some of the members of the chemical profession who may have forgotten their physical chemistry. Possibly, he has a little over-emphasised this side of the subject, but, at least, he makes ample amends in the sections on applied chemistry, which constitute roughly three-fourths of the book. Indeed, it may be confidently predicted that if a reader gets through the first few chapters, he will assuredly finish the book, for its interest increases as the chapters proceed.

This edition has been improved by the addition of numerous illustrations, and of suggestions for further reading at the end of each chapter, but the author has resisted the temptation to add another chapter on the rather hackneyed subject of vitamins.

A very wide field is covered as thoroughly as the nature of the work justifies, the information is up to date, and there is a gratifying absence of mistakes and mis-statements. On the latter score, it may, perhaps, be objected that some of the "chatty" passages, in which almost every chapter abounds, tend to emphasise

disproportionately the use, for some particular purpose, of the substance described, but this is hardly avoidable in such a rapid survey. It is questionable whether even a better book could not have been compiled by treating less subjects more fully. For example, the enormous subject of synthetic chemistry is discussed in 20 pages, embracing synthetic dyes, drugs, perfumes and resins!

Despite these minor criticisms, however, Professor Findlay's book can be unhesitatingly recommended to the enquiring layman. Every chemist who has a genuine love for his science, and deplores the lack of knowledge and sympathy displayed by the ordinary person, should make a point of giving a copy to his lay friend.

E. L. SMITH

**DIE BESTIMMUNG UND BEDEUTUNG DER WASSERSTOFFIONEN KONZENTRATIONEN IN DER GERBEREI.** By H. MACHON. Pp. 50. Freiberg: Buchdruckerei Ernst Mauckisch. (Privately printed.)

Modern views on the chemistry and physics underlying the wet work of a tannery are gradually but steadily being assimilated by the practical manager. Dr Machon's clearly arranged little book will be of considerable help in this direction.

The book is divided into a theoretical and a practical part. The theoretical part deals with the definition of acids and bases on the ionic theory, dissociation constants, the origin of the symbol  $pH$ , the meaning of "buffers," and a discussion of the characteristics of weak and strong acids and bases. In a description of the method of measuring  $pH$  values by the hydrogen electrode, attention is given very briefly to the numerous pitfalls which may arise. In cases where a solution is actually changed by the hydrogen, the quinhydrone electrode is recommended if the  $pH$  value of the solution is not over 9. From such very general statements as these, it will be seen that, as the author clearly states in the Introduction, the book is not intended to compete with the existing excellent standard books on the subject. A table of indicators and a list of buffer solutions covering the  $pH$  range 1.3 to 10.0 is given, and there is also a reference to the tinted gelatin disc colorimeter of Wulff. A half page is devoted to the errors of Indicator methods, and the composition of a "Universal" indicator is given. There is also a chapter on the behaviour of proteins, the iso-electric point, and the effect of the presence of salts on acid-plumping.

In the practical part each process is discussed from the point of view of limiting  $pH$  values are given for tannery waters and for the liquors employed in soaking, liming, deliming, bating, tanning (by the vegetable and the chrome process) bleaching and fat liquoring. Some of the values given are somewhat surprising; for instance, a water of 14° German hardness is quoted as having a  $pH$  value of 6.2; lime liquors, both sharpened and unsharpened, are said to vary in  $pH$  value from 11.5 to 12.1, and the maximum point for degree of plumping of hide is given at  $pH$  4. It is emphasised that determination of the  $pH$  value is no substitute for tannin analysis in the control of tan liquors, and that it is not to attach too much importance to  $pH$  value only, although it is in itself an invaluable means of control.

R. F. INNES





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